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EXPERIENCE WITH THE HORMONE TEST FOR PREGNANCY*

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There seems little if any need to emphasize the utility of a relatively reliable laboratory test for pregnancy as a diagnostic aid. Aside from furnishing a procedure to diagnose normal pregnancy at a much earlier date than is possible from the usual clinical signs, the hormone test for pregnancy serves particularly in those instances where early interference with pregnancy is indicated, as in some cases of diabetes, tuberculosis, renal disease, or cardiac lesion. Even more important perhaps is its service in the differential diagnosis of more or less acute abdominal conditions in which normal pregnancy, ectopic gestation, toxemia of pregnancy, hydatid mole or chorio-epithelioma, in other words conditions which yield a positive reaction, are possible factors. Finally it also serves as a test for the completeness of removal of the products of gestation, hydatid mole, or malignant growths originating from chorionic villi.

Current medical literature contains the records of a rapidly increasing number of statistical reports on the use of the test in the diagnosis of pregnancy as well as of case histories showing complex conditions which were clarified by the knowledge from the test of the presence or absence of a tubal or other extra-uterine conception.

As in the case of any other clinical laboratory test, its value is in direct ratio to its reliability and the proportion of errors, incorrect positives being more annoying and in some instances more hazardous than incorrect negatives.

The result of a number of tabulations by various authors shows

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a striking similarity in the conclusion that the test under consideration is correctly positive in as high as 98 per cent of normal pregnancy cases. On the other hand there is no similarity in the experience with incorrect positives. A number of reports by others as well as our own results fortunately show no false positives. Other workers report a small number at most and account for them in one way or another, leaving relatively few in which the positive result could not be explained. In ectopic gestation the results in the different reports are not so similar as would be desirable, some showing as low as 50 per cent positive while others report a higher percentage of positive results. While it is generally stated that the death of the fetus is promptly followed by negative reactions, it is also reported by Wilson and Corner² and others that positive tests were obtained in cases where the dead fetus was retained for a considerable period.

No definite decision seems to have been reached as yet as to the shortest duration of pregnancy recognizable by a positive reaction but there are many reports quoting positive tests two or three days after the first day of the first missed menstruation, though this of course does not establish the date of conception. In few instances where pregnancy could be referred to a single date, a positive reaction has been reported in from ten to fourteen days after the coitus. Jones and Mugrage² report one case in which a positive reaction was obtained ten days after coitus. Wilson and Corner call attention to their experience of some negative reactions during the first month of pregnancy where positive reactions were obtained in from four to seven days later. It has generally been found that the reaction is most constant and pronounced during the early part of pregnancy and perhaps less so during the later months, persisting however until one or more days after delivery. Wilson and Corner claim that with the Friedman modification the positive reaction persists for twenty-four to seventy-two hours postpartum while with the Aschheim-Zondek test this period is from seven to eight days.

For the original Aschheim-Zondek test four or five immature white mice serve the purpose while for the Friedman modification one mature female rabbit is used. The advocates of the Fried-

man modification emphasize the difficulty attending the securing of a large number of mice of proper age at all times compared with the ease of having on hand a number of grown female rabbits properly segregated. Also that the Friedman test is complete in forty-eight hours while the Aschheim-Zondek technic requires 100 hours. The advocates of the Aschheim-Zondek method claim it to be a bit more sensitive and somewhat less liable to error in reading the result.

The first urine passed in the morning is the most desirable specimen for the test, taken at a time when the patient is not under medication. Catheterization is not necessary but a clean specimen in a clean container is essential. Many drugs taken by patients have an undesirable effect on the animal used for the test and some are promptly fatal to it. Several methods have been devised for the preliminary removal of objectionable qualities and others for concentrating the hormone in the urine to lessen the amount of specimen necessary to inject into the animal and to produce a more pronounced and possibly earlier result.

While the direct object of this communication is to detail our experience with the hormone test for pregnancy, it seemed desirable to present the above preamble as a premise. It is not proposed at this time to enter into a discussion of the advantage of one procedure over another or even to advocate refinements in the test. It must be apparent to all in this work that niceties of technic and improvements in the details of the test must result in still greater accuracy and possibly in the saving of time, though such modifications still require the test of further experience.

The following report concerns 1048 hormone tests for pregnancy in cases of various kinds in all of which a positive clinical diagnosis at least in so far as pregnancy is concerned was originally or subsequently established. We are indebted to Drs. F. C. Holden, F. W. Rice and H. C. Williamson of the obstetrical and gynecological services of Bellevue Hospital for a large part of the material. In 487 cases the original Aschheim-Zondek test and the Friedman modification were both done with the same specimen.

The report includes the technic used, a complete table of all tests done and the comparative results with the two tests in cases

of normal and abnormal pregnancy and allied conditions, of uterine and tubal disease without pregnancy and in beginning menopause, all cases offering differential diagnostic problems: also in acromegaly on account of the possibly associated endocrine activity. Our experience with the test during the first half of pregnancy is detailed. There is also a consideration of the mortality of the animals during the test period leading to the loss of the test, and finally there are conclusions prompted by our study.

In table 1 there is a list of all the tests we have made with the Aschheim-Zondek or the Friedman method of procedure. The

TABLE 1
COMPLETE LIST OF HORMONE TESTS FOR PREGNANCY

| DIAGNOSIS | NUMBER OF TESTS | ASCHHEIM-ZONDEK OR FRIEDMAN TEST | | CORRECT per cent |
|---|-----------------|-------------------------------------|----------|-------------------------|
| | | Positive | Negative | |
| Normal pregnancy | 764 | 716 | 48 | 93.7 |
| Ectopic gestation | 80 | 68 | 12 | 85 |
| Hydatid mole | 6 | 6 | 0 | 100 |
| Beginning menopause | 63 | 0 | 63 | 100 |
| Fibromyoma uteri | 33 | 0 | 33 | 100 |
| Salpingitis | 62 | 0 | 62 | 100 |
| Amenorrhea unknown cause (no pregnancy) | 24 | 0 | 24 | 100 |
| Acromegaly | 16 | 0 | 16 | 100 |

number of instances of a negative result in pregnancy cases was about 6.3 per cent which figure is due largely to conservatism particularly in our early work with the test and which probably also explains the fact that an incorrect positive was not found in a single specimen examined. It was this result however that prompted doing the test by both methods on the same specimen and this produced a materially higher degree of accuracy with again no incorrect positive reactions as will be shown later.

An analysis of the comparative results according to the duration of pregnancy shows the following in our series:

| | PER CENT POSITIVE | |
|--------------------------|-------------------|----------|
| | Aschheim-Zondek | Friedman |
| First month or less..... | 91 | 94 |
| Second month..... | 93 | 93 |
| Third month..... | 95 | 90 |
| Fourth month..... | 87 | 94 |
| Fifth month..... | 99 | 94 |

The earliest pregnancy in this series in which a positive result was obtained was one in which conception could not have occurred longer than 14 days prior to the test and the next earliest where the test was made two days after the first day of the expected menstruation. The technic used in most of the work with the Aschheim-Zondek test was that as originally recommended, namely four or five immature white mice weighing 8 to 12 grams each were injected with respectively 0.2, 0.25, 0.3, 0.3, 0.4 cc. of urine twice a day for three days and killed at the end of 100 hours after the first injection. In a number of instances more recently we have injected the mice with the same or slightly smaller amounts three times the first day, twice the second day, and once the third day, according to a published recommendation. Two were killed seventy-two hours after the first injection. If negative, in no instance as yet were the remaining mice positive at the end of the usual 100 hours after the first injection. In the Friedman modification, 10 cc. of urine were injected into the marginal ear vein of a female rabbit about three to four months old and the animal autopsied forty-eight hours later.

The specimen used for the test was the first morning urine with previous instructions that no drugs should be taken by the patient during the preceding twenty-four hours. Clean specimens in clean bottles were generally obtained and aseptic collection was not demanded. Alkaline specimens were acidified and all were placed in an ice-box for several hours and then filtered before use. Specimens from a distance had one drop of cresol added to each 50 cc. of urine. The method recommended by Zondek for the removal of toxic substances by shaking the specimen with ether with or without the subsequent addition of glucose was not used in

the series now reported, nor was any attempt at concentration of the hormone made as also suggested by Zondek⁴ and by Eberson.¹ These improved methods appear trustworthy and interesting and will doubtless have to do with the ultimate refinements in the procedure.

The animal mortality during the test period is of interest and though probably avoidable with the recent refinements in technique, is still embarrassing when it occurs. In our series of cases twelve sets of mice and ten rabbits died during the test period, a mortality of 3.59 per cent and 3.17 per cent respectively. In the series when the test was done concurrently with both methods,

TABLE 2

COMPARATIVE ANALYSIS OF 487 COMPLETED TESTS WITH BOTH THE ASCHHEIM-ZONDEK AND FRIEDMAN METHODS

| DIAGNOSIS | TOTAL | ASCHHEIM-ZONDEK TEST | | | FRIEDMAN MODIFICATION | | | BOTH TESTS | | |
|--|-------|----------------------|----------|----------|-----------------------|----------|----------|-----------------|---------------|----------|
| | | Positive | Negative | Correct | Positive | Negative | Correct | Either positive | Both negative | Correct |
| | | | | per cent | | | per cent | | | per cent |
| Normal pregnancy | 365 | 343 | 22 | 94 | 339 | 26 | 92.9 | 358 | 7 | 98.1 |
| Ectopic gestation | 38 | 33 | 5 | 86.8 | 31 | 7 | 81.6 | 35 | 3 | 92.1 |
| Hydatid mole. | 3 | 3 | 0 | 100 | 3 | 0 | 100 | 3 | 0 | 100 |
| Tests in nonpregnant cases (see table 1). | 81 | 0 | 81 | 100 | 0 | 81 | 100 | 0 | 81 | 100 |

the mice and rabbit both died in only six instances, a mortality of 1.1 per cent which is negligible. In practically all cases these animal deaths were referable to decomposition or bacterial growth in the specimen, presence of acetone and diacetic acid, or to the use of drugs by the patient such as ergot, quinine, arsenic, luminol, morphine, codeine or aspirin.

In the interest of greater accuracy as previously stated, we undertook a series of 487 cases in which the Aschheim-Zondek and the Friedman modification were used concurrently, with results as detailed in table 2. These figures demonstrate the advantage of using both methods by the appreciably higher per-

centage of correct positive reactions in normal pregnancy and particularly in cases of ectopic gestation, a complete absence of incorrect positive reactions and a minimal loss of animals during the test period. Thus the combined methods show 98.1 per cent positive reactions in normal pregnancy compared with 94 per cent Aschheim-Zondek alone and with 92.9 per cent Friedman modification alone. In ectopic gestation there was a similar improvement, 92.1 per cent with the combined method compared with 86.8 per cent Aschheim-Zondek alone and with 81.6 per cent Friedman modification alone.

CONCLUSIONS

It was demonstrated in a relatively large series of cases that incorrect positive results can be avoided. The concurrent use of the Aschheim-Zondek test and the Friedman modification results in a higher proportion of correct positive reactions and a lessened liability of loss due to the death of animals during the test period. For these reasons the combined test is advocated until additional refinements make it unnecessary, particularly in cases presenting difficulties in differential diagnosis in which surgical intervention may be indicated.

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THE RESULTS OF TWO YEARS EXPERIENCE WITH THE FRIEDMAN TEST*

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Two years ago we reported before this society a series of fifty clinical cases in which the Friedman modification of the Aschheim-Zondek test for pregnancy had been used with gratifying results.⁹ This series was extended to 150 cases in a subsequent paper,¹⁰ and last year had reached a total of 390 tests, of which 278 had been checked by the subsequent clinical course.¹¹ The present report is a summary of our experience in approximately 1000 tests, of which 865 have been checked by subsequent clinical course. This marked numerical increase in the utilization of the test is further demonstrated in that our first series represented six months' work with repeated requests to clinicians for coöperation. During the past year we have received without solicitation over 600 specimens for diagnosis, and splendid coöperation in the reports of the subsequent clinical course.

This experience has been duplicated by numerous individuals who have carefully investigated the test, and may also be illustrated in the tremendous increase in the number of papers reporting experiences with the test.

In the preparation of our first report of cases, Friedman's fundamental work⁵ demonstrating the scientific principles of the test was the only available reference to this method, although we have since ascertained that Jares⁷ had confirmed Friedman's work. The reports of experience with the test since then have become quite numerous, and the value of the test has been demonstrated in thousands of cases.

Furthermore the evolution of titles under which the Friedman

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test has been reported, while numerous and varied, were logically standardized in the report of Magath and Randall.⁵ The active principle in the urine of pregnant women which will induce a characteristic change in the ovaries of immature female white mice, and its absence in practically all other conditions, represents the fundamental fact of the test, and was demonstrated, amplified, standardized, and popularized by Aschheim and Zondek. Friedman demonstrated that the ovaries of the rabbit might likewise be used as a biological indicator of the presence of this principle if the urine was injected intravenously. This represented a decided advance in the technique of the test. Therefore all tests in which the rabbit is the animal used as the biological indicator originate from the work of Friedman.

Another factor which has been responsible for the variety of names under which the Friedman modification has been reported, is the number of investigators who began working on the test as soon as Friedman's original paper concerning the availability of the rabbit appeared. It is pertinent that in Friedman's first papers no standardized technique or details of the actual test were proposed. Therefore each investigator was confronted with the necessity of working out his own technique. Schneider¹² was impressed with the maturing factor of the active principles in the urine of pregnant women as demonstrated originally by Aschheim and Zondek and therefore began using immature female rabbits. Friedman,⁵ having suggested the substitution of the rabbit for the mouse, was apparently influenced by the original technique employed with mice and therefore made repeated injections and examined the ovaries of the rabbits at autopsy. This technique is undoubtedly best for technicians who are not familiar with animal experimentation. Wilson and Corner,¹³ with long experience in the physiology of reproduction, examined the ovaries by laparotomy sixteen hours after injection with excellent results.

Our experience has passed through various stages prompted by expediency. Our present technique more nearly approaches that of Wilson and Corner than any other.

METHOD

We have always preferred adult rabbits weighing four pounds or more. However, occasionally there are younger rabbits in the lots we have received, and our results with these are as good as with those obtained from larger rabbits. In our earlier injections we used 5 cc. of urine intravenously and operated on the rabbit under anaesthesia eighteen to twenty-four hours later. If examination of the ovaries revealed negative results we reinjected 5 cc. of urine and reoperated after an additional twenty-four hours. In an occasional case we discovered a delayed reaction, but with more careful original examination of the ovaries we believe these could have been diagnosed. In the hope of avoiding a delayed reaction and obtaining a more decisive end result, we now inject 10 cc. of urine and routinely wait forty-eight hours; this eliminates approximately 50 per cent of our operative procedure, which is necessitated by the large increase in the number of specimens submitted for examination. Furthermore, since we routinely utilize all negative rabbits for subsequent tests until a positive reaction is obtained, we save our rabbits from undue trauma. Rabbits presenting a negative test may be immediately reinjected; if the urine is positive, they will react as well as animals which have had no injection. This means a saving of more than 50 per cent in animals. They may be used in this manner until they appear sick or develop troublesome infection or adhesions. Usually this method does not permit more than three successive tests on one rabbit. Occasionally when short of animals we reinject positive reacting rabbits, if seven days have elapsed following the previous operation. These cases are then subsequently controlled by injection of the same urine into a fresh rabbit when the new lot arrives. While we have encountered no errors by this method, we do not advocate its usage except in emergency. In these cases, previous to the injection, we open the abdomen and note the number, size, and distribution of hemorrhagic follicles from the previous test; forty-eight hours after this injection the ovaries are carefully compared with their previous condition. Positive cases are easily diagnosed; negative cases require careful consideration.

Examination of the ovaries of the rabbit by operation rather than autopsy has proved highly satisfactory. The animal is strapped to the board, anaesthetized with ether, the foot of the board is elevated so the intestines and oviducts drop toward the diaphragm, the hair is clipped short in the lower portion of the abdomen, and an antiseptic solution applied in this area. The abdominal incision is about two inches long, the oviducts are picked up with forceps and pulled down until the ovaries are visible. After examination the wound is closed with silk in two layers, an antiseptic reapplied to the wound, and the rabbits are placed in their cage without any dressing. The entire procedure is carried out by one man, rarely requires more than fifteen minutes, and the animals universally do well. We have outlined these details in the evolution of our technique rather minutely, not because they are better than that of others, but to emphasize that apparent variations in technique are not material, but have been largely developed by experience or as a result of local conditions.

A more material variation in the test is reported by Brown,³ in which the blood serum of pregnant women is injected intravenously into female rabbits with examination by autopsy twenty-four to thirty-six hours later. We used blood serum in the place of urine in 1930 with a small series of cases, and in those cases found it as reliable as the intravenous injection of urine. We considered it more cumbersome than the Friedman modification, and clinicians expressed such a great preference for the urine test that we discarded the serum test.

Another modification of the original Aschheim-Zondek test is that of Davis and Ferrill⁴ in which immature female rats from twenty-five to thirty days of age are used rather than immature female mice. In our original paper² we noted our experience with "immature white rats was similar to that obtained with white mice," and so far as we were concerned, this technique gave no improvement as a practical clinical test over the original Aschheim-Zondek test. The accuracy of this modification, however, has been further demonstrated by Dr. E. P. Durrant¹⁴ of the Department of Physiology of Ohio State University. This method may be of value in some communities where the rabbit and mouse are not so available as the white rat. The local animal supply is certainly a factor in the determination of what modification of the original test may be used, and if the biological principle remains unchanged, the results should not differ materially.

The complete series of tests outlined in table 1 represent the work of our laboratory and the laboratory of White Cross Hospital of Columbus, Ohio.

In any test where the check of accuracy or control depends upon the interpretation of a number of different individuals, the opportunity for error is undoubtedly increased. If, in addition, we appreciate the subsequent varied course which may present itself to the clinician for interpretation, correlation, and judgment as to the accuracy of the diagnosis submitted from this test, the possibility of error must likewise increase. For example, a married woman went to a clinician complaining of two missed periods and desiring to know if she was pregnant. Although she had been married seven years, examination revealed an intact hymen.

Further examination revealed the early usual presumptive signs of pregnancy. A specimen of urine submitted for examination gave a positive test. Later the woman went to another physician for puerperal care. The uterus was enlarged to about a three or four months pregnancy, but as time went on the size did not increase. Finally about the seventh month of her supposed pregnancy a second specimen of her urine was received for examination which proved to be negative. The physician concluded the first test had been in error and that the patient had a tumor

TABLE 1
SUMMARY OF RESULTS

| | | |
|--|-------------|---------|
| White Cross Hospital..... | 392 | } 1,002 |
| Department of Pathology, Ohio State University..... | 610 | |
| Subsequent clinical history accurately reported..... | 854 | } 865 |
| Subsequent clinical history equivocal..... | 11 | |
| Subsequent clinical history absent..... | 137 | |
| } 1,002 | | |
| <i>Tests checked by subsequent clinical course</i> | | |
| Test reported positive..... | 423 | } 865 |
| Test reported negative..... | 442 | |
| False positive tests..... | 5 or 1.20% | error |
| False negative tests..... | 15 or 3.40% | error |
| Equivocal tests..... | 11 | |
| Maximum error of series..... | 31 or 3.59% | |
| Minimum error of series..... | 20 or 2.32% | |
| Hydatid moles..... | 3 | |

in her pelvis. The patient refused any operative investigation; near the end of the eighth month of her supposed pregnancy she went into labor and spontaneously delivered macerated twins estimated to have been of about three months development. A lack of the complete history would have branded one of the tests as incorrect. Fortunately such cases are rare. In eleven of the cases cited in the table subsequent clinical history was such that the clinicians were unwilling to state whether or not the test had been correct. Of these six were positive and five were negative. They were followed either by atypical menstruation or abortions.

Less careful physicians in some of the other cases must have encountered the same thing and interpreted it according to the report which they had received. In 148 cases the subsequent clinical history is not known.

We encountered five false positive reactions and three hydatid moles,¹ which gave positive reactions. Of the false positive reactions one was diagnosed menopause, one an ectopic pregnancy which did not exist as proved by operation, the uterus as well as the tubes being normal, one as gall bladder disease, and two presumably functional amenorrhea.

The false negative reactions have constantly been more frequent in our experience than the false positive reactions. In fact in the first 130 positive reactions which we obtained there was not one in error. Bland, First, and Roeder² obtained a correct negative in 93.2 per cent and a correct positive in 82.4 per cent with the original Aschheim-Zondek test. With the Friedman modification we have obtained a correct negative in from 95.5 per cent to 96.6 per cent, and a correct positive in from 97.4 per cent to 98.8 per cent according to whether or not we include the eleven questionable cases as in error. We have insisted upon no report on the test being made positive unless it is unquestionably positive. This probably accounts for the larger number of false negatives in our series. We feel that these results are significant in that they represent a transition from a biological problem to a standard clinical test without undue variation in accuracy.

SUMMARY

1. The rapid and widespread usage of the Friedman test is briefly outlined.
2. No significant variation in Friedman's technique has appeared.
3. The transition from the stage of biological trial to a standard clinical test has been accomplished without significant variation in accuracy.

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THE COMPARATIVE VALUE OF MONOCHLORBENZENE AND THYMOL WHEN USED WITH FLUORIDE AS PRESERVATIVES OF BLOOD FOR CHEMICAL ANALYSIS.

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The sugar of the blood undergoes such a marked reduction in concentration beginning soon after the blood is drawn that delayed analyses yield worthless results. Moreover, the ammonia content increases with the standing of blood and, since total non-protein nitrogen and urea are determined as ammonia, erroneous figures for these constituents are obtained when analyses are not made on fresh blood. For these reasons, the desirability of finding methods for rendering these blood chemical constituents more stable, so that the analyses may be delayed to a convenient time, has been the incentive for considerable investigation in recent years. Many of the methods suggested have proven more or less satisfactory, but there is a lack of confirmation of the efficacy of any single preservative for all of the non-protein nitrogenous constituents and sugar. The literature on the subject is extensive and a brief review will serve to show which methods have thus far proved most successful.

Among the first preservatives suggested was formaldehyde, which was used by Denis and Aldrich¹ to prevent glycolysis. However, Bock² reported that only a very few brands of commercial formaldehyde were suitable for this purpose, the majority causing an appreciable reduction of the Folin-Wu sugar reagent. Splatt²⁴ likewise found formaldehyde to be unsatisfactory for preventing a change in the sugar concentration, as there was a continual increase in sugar or other reducing substances in the blood up to seven days, at which time coagulation of the specimen occurred.

Picric acid was suggested by John¹⁰ as being successful in the dual capacity of protein precipitant and preservative for sugar. Obviously, this substance could not be used when sugar was to be determined by other methods than those employing picric acid.

Potassium fluoride used alone was found satisfactory for the preservation of sugar and carbon dioxide combining power for seven days by Major¹⁵ (5) when the specimen was collected and stored in a sterile manner. Denis and Beven⁵ reported that sodium fluoride, when added to oxalated blood, acted as an efficient preservative of sugar for ninety-six hours and of other constituents for forty-eight hours. Roe, Irish, and Boyd¹⁶ also studied the preservative action of fluoride and found that 10 mgm. of sodium fluoride per cubic centimeter of blood prevented changes in non-protein nitrogen, uric acid, creatinine, sugar and cholesterol in sterile blood for at least ten days, but that satisfactory preservation was not obtained even with 20 mgm. of sodium fluoride per cubic centimeter of blood under non-sterile conditions.

Sander²² proposed sodium fluoride and thymol in amounts of 0.02 gram of the former and 0.002 gram of the latter per cubic centimeter of blood and found it to be an efficient general preservative for a period of five to six days. The effectiveness of this method has been amply verified by other workers and has practically come to be the standard procedure. Denis and Hume⁶ found it satisfactory in preventing a destruction of the sugar for five to six days, while Cameron and Williamson³ reported it efficient for the nitrogenous constituents as well as for sugar for a period of three to four days. Nadler, Starr, and Tukey¹⁷, using this combination of thymol and sodium fluoride, found it suitable for the preservation of sugar for six to fourteen days, and John¹¹ found it more satisfactory than other methods for the same purpose. Splatt, using this method to prevent glycolysis, found that the concentration of the sugar was maintained practically at a level up to fourteen days when the determinations were performed by the Maclean method. Schwenker²³ reported thymol-fluoride to be an efficient preservative for

sugar in one-half the amounts advised by Sander when the blood is analyzed according to the Folin-Wu procedure, but that the sugar values, when they are determined by the Lewis-Benedict^{14, 15} method, are from 7.5 to 70 per cent too high. In this article, Schwentker also stated that he was able to confirm the results of Sander on the efficacy of thymol-fluoride in preventing changes in the concentration of the nitrogenous blood chemical constituents. Kilduffe and Springer¹² reported that thymol and sodium fluoride as used by Sander interfere with urea determinations by the Folin-Wu technic. Rose and Schattner²⁰ found that blood preserved with sodium fluoride and thymol lost 50 per cent of the sugar in three days and all of it in six days. Thus, the literature shows a lack of uniformity of opinion concerning the preservative qualities of thymol and sodium fluoride.

Rose and Schattner obtained excellent preservation of sugar with a mixture of monochlorobenzene and sodium fluoride. By this preservative combination, the blood sugar was kept within 0.015 per cent of its original value for fifteen to twenty days, and in two instances up to seventy-two days with no greater variation. However, this report contained no mention of the influence of these combinations of substances on the preservation of the nitrogenous blood chemical constituents.

In addition to the method just mentioned, Rose and Schattner²¹ recently outlined a procedure particularly adapted for sugar preservation in samples for use in sugar tolerance tests. Blood samples of 0.1 to 0.2 cc. were stirred into a desiccated mixture of 15 grams of sodium sulfate and 200 mgm. of sodium fluoride contained in small vials. Samples thus dehydrated gave tests within 2 to 5 per cent of the initial analysis from a few days after collection up to three months.

The purpose of the present investigation was: (1) to find a method for the preservation of the nitrogenous blood chemical constituents as well as the sugar when the blood specimens are sent through the mails, and (2) to find some material which could be added to oxalated bloods to preserve effectively all of these constituents for at least two days when the blood is stored in an ice box.

EXPERIMENTAL

Human blood was used in all of our investigations, and the blood specimens were collected without regard to the time of day or to the length of time elapsed since the last food. No effort was made to employ sterile syringes or containers. By means of a Luer syringe, 10 to 20 cc. of blood were drawn from a cubital vein and transferred immediately to a 50 cc. Erlenmeyer flask, or to a wide mouthed bottle of the same capacity in which the anticoagulant and preservative agents had previously been placed. The anticoagulant was distributed in the bottles by adding the required volume of a solution of known strength of potassium fluoride and then evaporating off the water. The remaining compound of the preservative combination, the thymol or monochlorobenzene, was then placed in the containers just previous to the addition of the blood.

For no particular reason, we employed potassium fluoride in our studies instead of the more customarily used sodium fluoride. In checks which were made later to determine the comparative action of the two compounds, no difference in preservative ability was found. Neither was there any apparent difference in their respective action toward the analytical reagents. It was thought possible that the comparatively large amount of potassium in the form of potassium fluoride that was required to prevent clotting of the blood might interfere with the uric acid and creatinine determinations as suggested by Benedict,¹ but no such interference was ever discernable.

The analytical methods employed were for the most part those recommended by Folin and Wu^{2,3} as modified for use with the Peebles-Lewis colorimeter.^{15,16} The blood was precipitated according to the Folin-Wu procedure and analyses for sugar, total non-protein nitrogen, urea nitrogen, uric acid, and creatinine were performed on this filtrate. In the determination of uric acid, the reagent proposed by Folin and Trimble⁷ was used. In the micro-Kjeldahl determination for total non-protein nitrogen, the Koch-McMeekin method¹³ was employed. After sufficient blood had been reserved for an initial analysis, the stoppers of the containers in which the specimens were stored were sealed by dipping them in melted paraffin to prevent any possible evaporation of the liquid. At certain intervals the containers were unsealed, sufficient blood was withdrawn for analysis, and the containers were immediately resealed for further storage.

Several substances not heretofore suggested were investigated for possible powers of preservation. Chlorazene, hexylresorcinol, and furfural caused reduction of the copper-containing reagents to such a degree that they proved valueless. Menthol, while it had no deleterious effect on the reactions involved, failed to exhibit any preservative action.

While we were engaged in this attempt to find a new preserva-

tive agent, the report of Rose and Schattner²⁰ appeared with statements of the excellent results obtained by the use of monochlorobenzene and sodium fluoride in the preservation of sugar in the blood. A few tests in our laboratory with monochlorobenzene and potassium fluoride proved that not only was the sugar satisfactorily preserved, but that this combination prevented a change in other blood chemical constituents as well. Since monochlorobenzene gave evidence of such exceptional possibilities as a blood preservative material, we decided to abandon further search for a new substance and to give our attention to a comparative study of the preservative properties of monochlorobenzene and thymol on fluorided and oxalated bloods.

The first comparison consisted in determining the respective preservative properties of thymol-fluoride and monochlorobenzene-fluoride on blood stored at room temperature (25°C.). The results obtained showed no superiority of one preservative method over the other. Consequently, the detailed tabular report of 113 determinations on eight blood specimens was not deemed of sufficient worth to be included in this report. However, the results may be summarized as follows:

With thymol-fluoride the preservation of sugar was satisfactory for six to eight days, and in one case for twenty-one days. The maximum period of satisfactory preservation for the nitrogenous blood chemical constituents proved to be six to eight days. Monochlorobenzene-fluoride held the blood sugar content without appreciable change for seven to twenty-six days, thereby confirming the report of Rose and Schattner.²⁰ Non-protein nitrogen remained practically constant up to seven days, and the variation up to fourteen days was not large. The results for urea-nitrogen were nearly as satisfactory, and the preservation of uric acid and creatinine were excellent for even longer periods.

A second comparison consisted in an attempt to preserve blood while storing specimens in an incubator at a temperature of 37°C. Under these conditions there was a failure in preservation of the blood specimens with the use of either the thymol-fluoride or the monochlorobenzene-fluoride combination. During

these tests conducted at elevated temperatures, clots or coagulum appeared in the specimens preserved with monochlorobenzene-fluoride but were absent in specimens containing thymol-fluoride.

One other means of obtaining comparative results was tried, that of shipping the blood specimens through the mail and thereby making tests under conditions which the bloods would actually undergo, were they to be sent to the laboratory from surrounding localities. The specimens were collected and placed in 50 cc. bottles as in previous experiments. After sufficient blood was

TABLE 1

PRESERVATIVE EFFECT OF THYMOL-FLUORIDE ON BLOODS SHIPPED THROUGH THE
MAILS*

(Thymol, 0.02 gram and potassium fluoride, 0.275 gram per 20 cc. of blood)

| SPECIMEN NUMBER† | GLUCOSE | | NON-PROTEIN NITROGEN | | UREA NITROGEN | |
|------------------|---------------------|---------------------|-------------------------|---------------------|---------------------|---------------------|
| | Initial analysis | Final analysis | Initial analysis | Final analysis | Initial analysis | Final analysis |
| | mgm. per 100 cc. | mgm. per 100 cc. | mgm. per 100 cc. | mgm. per 100 cc. | mgm. per 100 cc. | mgm. per 100 cc. |
| 61 (6 days) | 95 | 70 | 32 | 39 | 15 | 17 |
| 63 (6 days) | 88 | 67 | 42 | 47 | 18 | 21 |
| 40 (7 days) | 98 | 99 | 31 | 38 | 14 | 19 |
| 42 (8 days) | 92 | 78 | 30 | 40 | 14 | 15 |
| 55 (9 days) | 90 | 88 | 26 | 34 | 12 | 17 |
| 59 (9 days) | 89 | 72 | 26 | 37 | 12 | 18 |

* Figures for uric acid and creatinine were omitted from this table to conserve space. Practically no change occurred in these constituents.

† Figures in parentheses represent the number of days elapsing between initial and final analyses.

removed for an initial analysis, the containers were carefully packed in mailing tubes and sent to various destinations which afforded journeys of from four to twelve days, as indicated in tables 1 and 2. In most cases, identical bloods were used for testing both preservative combinations. This was accomplished by drawing 20 cc. of blood from each patient and discharging 10 cc. into each of two bottles containing the respective preservatives. After removing sufficient blood for immediate analysis, these pairs were mailed and upon their return a second analysis was performed.

The experimental results under actual mailing conditions are given in table 1 for thymol-fluoride and in table 2 for monochlorbenzene-fluoride. Comparing the figures of the two tables, it will be seen that with few exceptions the thymol-fluoride composite failed to preserve the blood chemical constituents effec-

TABLE 2
PRESERVATIVE EFFECT OF MONOCHLORBENZENE-FLUORIDE ON BLOODS SHIPPED
THROUGH THE MAILS*
(Monochlorbenzene, 0.2 gram and potassium fluoride, 0.275 gram per 20 cc.
of blood)

| SPECIMEN NUMBER† | GLUCOSE | | NON-PROTEIN NITROGEN | | UREA NITROGEN | |
|------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | Initial analysis | Final analysis | Initial analysis | Final analysis | Initial analysis | Final analysis |
| | <i>mgm. per 100 cc.</i> | <i>mgm. per 100 cc.</i> | <i>mgm. per 100 cc.</i> | <i>mgm. per 100 cc.</i> | <i>mgm. per 100 cc.</i> | <i>mgm. per 100 cc.</i> |
| 72 (4 days) | 85 | 81 | 42 | 46 | 16 | 17 |
| 73 (4 days) | 86 | 85 | 48 | 49 | | |
| 74 (4 days) | 85 | 85 | 41 | 42 | 16 | 16 |
| 75 (4 days) | 86 | 84 | 48 | 50 | 17 | 18 |
| 60 (6 days) | 85 | 80 | 34 | 34 | 17 | 17 |
| 62 (6 days) | 92 | 89 | 42 | 42 | 18 | 19 |
| 41 (7 days) | 96 | 98 | 32 | 34 | 16 | 16 |
| 43 (8 days) | 92 | 90 | 30 | 31 | 15 | 15 |
| 64 (8 days) | 82 | 77‡ | | | | |
| 65 (8 days) | 88 | 80‡ | | | | |
| 66 (8 days) | 89 | 84‡ | 34 | 41‡ | | |
| 68 (12 days) | 98 | 96 | 34 | 36 | 14 | 17 |
| 69 (12 days) | 98 | 95 | 34 | 38 | 14 | 17 |
| 70 (12 days) | 90 | 92 | 33 | 35 | 14 | 16 |
| 71 (12 days) | 89 | 91 | 33 | 39 | 14 | 16 |

* Figures for uric acid and creatinine were omitted from this table to conserve space. Practically no change occurred in these constituents.

† Figures in parentheses represent the number of days elapsing between initial and final analyses.

‡ Specimens contained a large amount of clotted material upon return.

tively even for six days, whereas, except in the cases noted below, the monochlorbenzene-fluoride accomplished excellent preservation for as long as twelve days. Attention should be called to the lack of preservation by monochlorbenzene-fluoride that occurred in specimens #64, #65, and #66. Each of these

bloods contained a large amount of clotted material that resembled the condition resulting when the blood specimens of a previous series of tests were kept in the incubator, and only a small amount of fluid blood was available for analysis. These specimens showed a decrease in sugar content and specimen # 66, the only one of the three allowing sufficient fluid for an analysis of the nitrogenous constituents, gave an increased total non-protein nitrogen. This occasional failure of the monochlorbenzene-fluoride to preserve blood would be more serious, were it not for the fact that evidence of the deterioration is shown by the physical condition of the specimen (occurrence of clots) even before analysis is performed. In contrast to this, the blood specimens in which thymol-fluoride was used gave no evidence of failure of preservation by their physical appearance.

The following explanation might be offered as to why under laboratory conditions so little difference was found, and yet under mailing conditions so decided a divergence occurred, in the comparative powers of preservation of thymol-fluoride and monochlorbenzene-fluoride. No temperatures between 25°C. and 37°C. were tried in preliminary work in the laboratory. Although there is no means of knowing the conditions to which blood specimens are subjected in the mail, it may well be that temperatures higher than 25°C. but lower than 37°C. are experienced and that the monochlorbenzene-fluoride acts as a preservative under such conditions whereas the thymol-fluoride fails. We see then that, even though the results obtained at room and incubator temperatures show no superiority of the one preservative over the other, the data obtained under mailing conditions prove conclusively that monochlorbenzene-fluoride is vastly superior to thymol-fluoride as a preservative.

Throughout this entire study 0.2 gram (0.18 cc.) of the monochlorbenzene was used to 20 cc. of blood. While this amount is not completely miscible with 20 cc. of water, no difficulty was encountered in the soiling of pipettes or containers even when a larger amount was used with the same volume of blood. It is probable that any excess in the blood is removed in the process of protein precipitation and that the amount remaining in the

filtrate is merely in the form of a saturated solution and thus has no effect on the glassware. The minimum effective amount of monochlorbenzene for the preservation of all constituents was found to be 0.1 gram per 20 cc. of blood but, since no interference was found with 0.2 gram, we have used this amount routinely in order to allow an excess for a safety factor.

TABLE 3

PRESERVATIVE ACTION OF MONOCHLORBENZENE-OXALATE ON BLOODS STORED AT A TEMPERATURE OF 0° TO 6°C.*

(Monochlorbenzene, 0.2 gram and potassium oxalate, 0.04 gram per 20 cc. of blood)

| SPECIMEN NUMBER† | GLUCOSE | | NON-PROTEIN NITROGEN | | UREA NITROGEN | |
|------------------|------------------|------------------|----------------------|------------------|------------------|------------------|
| | Initial analysis | Final analysis | Initial analysis | Final analysis | Initial analysis | Final analysis |
| | mgm. per 100 cc. | mgm. per 100 cc. | mgm. per 100 cc. | mgm. per 100 cc. | mgm. per 100 cc. | mgm. per 100 cc. |
| 26 (2 days) | 86 | 88 | 28 | 30 | 15 | 17 |
| 30 (2 days) | 70 | 74 | 29 | 30 | 12 | 13 |
| 32 (2 days) | 102 | 97 | 27 | 26 | 13 | 12 |
| 34 (2 days) | 98 | 97 | 28 | 27 | 13 | 13 |
| 37 (2 days) | 105 | 103 | 30 | 28 | 13 | 13 |
| 38 (2 days) | 94 | 92 | 31 | 32 | 13 | 14 |
| 18 (4 days) | 93 | 87 | 27 | 28 | 17 | 17 |
| 19 (4 days) | 100 | 104 | 31 | 33 | 12 | 13 |
| 26 (4 days) | 86 | 88 | 28 | 29 | 15 | 16 |
| 32 (4 days) | 102 | 103 | 27 | 28 | 13 | 13 |
| 34 (4 days) | 98 | 98 | 28 | 27 | 13 | 13 |
| 37 (4 days) | 105 | 106 | 30 | 29 | 13 | 13 |
| 38 (4 days) | 94 | 91 | 31 | 30 | 13 | 14 |

* Figures for uric acid and creatinine were omitted from this table to conserve space. Practically no change occurred in these constituents.

† Figures in parentheses represent the number of days elapsing between initial and final analyses.

The other problem concerning which information was desired, as previously mentioned, was to find a preservative which when added to oxalated blood would, under conditions of refrigeration, prevent a change in the chemical constituents and thereby permit a postponement of analysis until a convenient time. Without such a preservative, it would be necessary to keep a technician

on duty in the blood chemistry laboratory on Sundays and holidays. On the other hand, if such a preservative material were known, it would be possible to take bloods on those days and store them in the ice box during the twenty-four to forty-eight hours until the next regular laboratory day, thereby making it unnecessary to conduct immediate analyses except when an emergency request was received. The relative efficacy of the two preservatives previously used, thymol and monochlorbenzene, were studied in this connection.

To our surprise, thymol when added to the oxalated bloods proved useless as a preservative. Even after so short a period of time as twenty-four hours none of the blood chemical constituents except uric acid and creatinine were maintained within satisfactory limits. The probable explanation of this failure is that at a temperature of 6°C. thymol is so slightly soluble in blood that no preservation could result. On the other hand, the addition to oxalated bloods of monochlorbenzene (0.2 gram per 20 cc. of blood) gave almost perfect preservation for as long as ninety-six hours. In table 3 it may be observed that without exception all constituents studied were preserved within narrow limits for this length of time. For longer periods than ninety-six hours preservation was not so perfect, but the shorter period was sufficient to fulfill the requirements desired.

SUMMARY

1. A combination of monochlorbenzene and potassium fluoride was found to be a more effective preservative for blood sugar, total non-protein nitrogen, and urea than thymol and potassium fluoride. Little change occurs in the uric acid and the creatinine with either preservative combination.

2. Blood specimens may be transported through the mails in journeys up to eight days with no appreciable change in either the blood sugar or the nitrogenous blood chemical constituents when a preservative combination composed of 0.275 gram of potassium fluoride and 0.2 gram of monochlorbenzene per 20 cc. of blood is employed. In journeys of twelve days, the sugar is well preserved, and only slight changes occur in the non-protein nitrogenous constituents.

3. Neither monochlorbenzene-potassium fluoride nor thymol-potassium fluoride will satisfactorily preserve blood specimens which are subjected to a temperature of 37°C. for twenty-four hours or longer.

4. If a blood specimen treated with monochlorbenzene-potassium fluoride has been subjected to high temperatures during passage in the mails, there will be a failure of preservation of the blood chemical constituents. Such blood will contain numerous small clots which will interfere with pipetting of the blood. The absence of such clots, therefore, offers a simple criterion as to successful preservation.

5. When preserved by the addition of 0.2 gram of monochlorbenzene to 20 cc. of blood, oxalated blood specimens may be stored in a refrigerator at 6°C. for ninety-six hours with practically no variation in the blood chemical constituents.

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EVIDENCE IN SUPPORT OF THE NEURO-EPITHELIAL NATURE OF MOLES*

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Moles or nevi of the skin are easily accessible for macroscopic and microscopic study, yet one hundred years after the development of the achromatic microscope, observers still disagree as to their true biologic nature. From a study of their histology two main schools have arisen, one contending that they are of mesodermal origin and the other that they are of ectodermal origin. Among the chief supporters of the mesodermal origin are, Virchow,³¹ Demiéville,⁷ Ehrmann,⁸ Ribbert,^{25, 26} Recklinghausen,²⁴ Lubarsch,¹⁷ Jadassohn¹³ and Soldan.²⁵ Unna, in 1892 propounded the ectodermal theory; this was at first strongly opposed, but later drew to its support such able investigators as Krompecher,¹⁵ Kromayer,¹⁴ Dalla Favera,⁵ Darier,⁶ Marchand,¹² Gilchrist,¹² Bloch^{3, 4} and Miescher.²³ In considering soft nevi or true moles Unna said: "All pigmented and nonpigmented, flat, just elevated nevi which are removed from infants and children, show a direct connection of the surface epithelium, and, in parts rich in follicles, of the prickle layer of the hair follicles, or even of the ducts of the coil glands, with the cellular cords of the nevus" (fig. 1).

Langerhans¹⁶ described certain cells in the epidermis which bear his name. He found these cells to be pear-shaped, situated three to five layers above the basal layer in the rete malpighii and to have several processes, one directed toward the dermis similar to an axone, the others toward the stratum corneum similar to dendrites. He noted that they never contained pigment and manifested a special affinity for gold chloride, which led him to conclude that they were of a nervous nature. He was of the

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opinion that they were not epithelial cells, nor could he find any connection between the single axone-like process and the tactile corpuscles in the dermis.

Richl, in 1884, discovered in the epidermis of man a dendritic cell; Bloch¹ described this cell in detail. Becker² also described the cell in a consideration of melanin pigmentation. The cell is situated in the basal layer and occasionally in the adjacent layers of the epidermis between the nondendritic cells. Its body, which

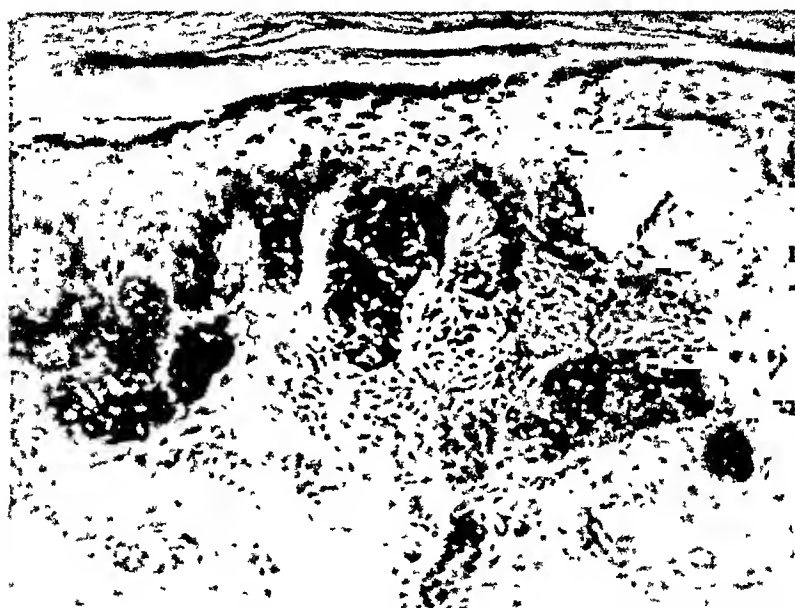


FIG. 1. PIGMENTED MOLE FROM THE TOE OF A YOUNG MAN.
This shows the origin of the nevus cells from the stratum germinativum.

is often irregular, contains varying amounts of melanin. The dendrites which vary from two to three or more also contain melanin, branch frequently, extend between the interstices of the cells and are said to communicate with one another. It is generally agreed that this cell is a true epithelial melanoblast, and unlike the Langerhans' cell is dopa positive and stains well with silver nitrate.

It was thought best to describe the Langerhans' and dendritic cells in the foregoing paragraphs since it is necessary to have a

clear conception of these two entities in order to understand the modification of the ectodermal theory of origin of moles which is to follow.

Simon²⁷ spoke of certain nevi as "nerve nevi" and thought that they were connected in some obscure way with the nervous system. This type of nevus was called by von Baerensprung,¹ "naevus unius laterus," and by Unna,^{29, 30} "naevus linearis." These nevi were thought to have some relationship to trophic nerves. As a matter of fact, they have practically nothing in common with true moles and should not be considered in the same category. Soldan,²⁸ pointed out that pigmented spots of the skin are frequently the first signs of a fibromatous process of the connective tissue of the nerves of the skin, and that the pigmentation is probably a consequence of this fibromatosis. He also pointed out that neurofibromas develop as the result of proliferation of the connective tissue of the peripheral nerves and that from the fibromatous processes of the pigmented spots, warts, soft fibromas, pigmented nevi, and so forth, are developed. According to him the cordons and nests of nevus cells described by Unna and others, are neither epithelial tumors nor lymphangiofibromas of Recklinghausen, but neurofibromas.

Masson^{19, 20} should be given full credit for bringing about a radical modification of the ectodermal theory of the origin of moles. It is his belief that they are formed essentially by the abnormal proliferation of the ends of the tactile nerves. He contends that they are not neurofibromas in the old sense that they arise by proliferation of the connective tissue intimately associated with nerves, as held by Soldan, but are composed of the syncytium of Schwann, the cells of which are partially neurotized. It is probable that the connective tissue of nerves referred to by Soldan is the same as that now spoken of as tissue of Schwann.

It seems pertinent here to call attention to an article written by Masson²⁰ on the Langerhans' cell in which he stated that the cell is capable of gold impregnation, which agreed with Langerhans' original description; however, he endowed it with properties which do not belong to it but do belong to the dendritic cell, namely, the ability to form melanin, as shown by Bloch's dopa reaction and

the staining of the melanin granules with silver nitrate. In other words, Masson created a hybrid cell out of the true Langerhans' and dendritic cells. The foregoing error, as previously pointed out by Bloch, has led to considerable confusion and no doubt has done much to discredit Masson's work on the nervous origin of moles.

It is Masson's belief that Langerhans' cells, or what he calls Langerhans' cells, the normal cells of Merkel-Ranvier and the epithelioid nevus cells are cells of the same nervous origin in which the function varies, and also that the cells of Langerhans as present in the normal epidermis are autonomous, or that they arise from, and give rise to Langerhans' cells and are not segregated and modified malpighian cells, as held by Unna, Kromayer and Darier. Masson further stated that a cellular nevus is able to grow by proliferation and differentiation of its own intradermal elements, without the epidermis participating. He observed a superabundance of neurites in nevi, and believed that their distribution among the nevus cells confirms the idea that the tumors correspond to a proliferation of nervous elements, and that since they present a form of differentiation comparable to tactile corpuscles, one can imagine that the nerves which ramify there are tactile. In his conclusions he stated that the epithelioid nevus cells are comparable to the cells of Merkel-Ranvier, the nevus corpuscles are comparable to the corpuscles of Wagner-Meissner, and that these two elements are associated in a syncytium with a system of plexiform and nonmyelinated nerve fibers attached to certain myelinated nerves of the dermis. It is his belief that all pigmented nevi are neuromi and that all are neuromatous terminations of tactile nerves. He gave two distinct points of origin of nevi, one epidermal from his so-called Langerhans' cell, and the other intradermal or nervous. It is his belief that the elements arising from these respective points come together and fuse in the dermis.

We were prompted to write this paper by the microscopic evidence revealed in the examination of a soft papillary pigmented mole, 1.5 cm. in diameter that was situated in the skin in the postero-auricular region of a woman aged thirty-one years. Just

beneath the epidermis, this mole presented the usual alveolar masses of nevus cells with spheroidal hyperchromatic nuclei and scant cytoplasm. These cells which to us are in an undifferentiated state are called epithelioid cells by Masson and are thought by him to be comparable to the normal cells of Merkel-Ranvier



FIG. 2. PIGMENTED MOLE OF SKIN OF POSTERIOR AURICULAR REGION

Note the alveolar masses of undifferentiated cells, high in the dermis and considered by Masson to be comparable to the cells of Merkel-Ranvier. Directly continuous with these cells are structures which are described in figure 3.

(fig. 2). Lying deeper in the dermis but directly continuous with the undifferentiated nevus cells are cord-like structures which contain fibrillated bodies, the so-called lames foliacees of Masson (fig. 3). These bodies are made up of fine fibrillary processes which intermingle with each other. Intimately associated with

them are a few scattered flattened nuclei. It is reasonable to assume that these processes represent the cytoplasm or a differentiated product of nevus cells. Wherever these fibrillated bodies occur, the nuclei are sparse and small in contrast to the nuclei of the undifferentiated nevus cells which are usually collected into

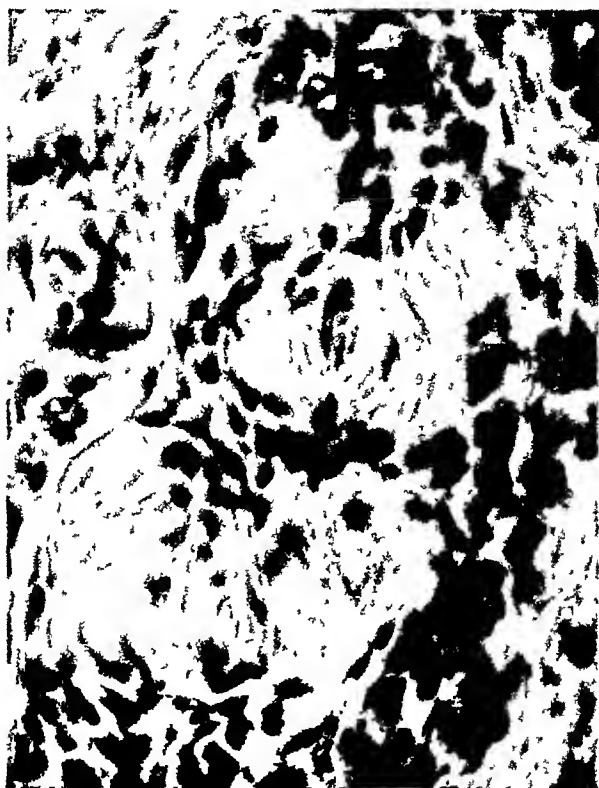


FIG. 3. HIGH POWER OF LAMBS FOLLICLES, CONTAINING A TUFT-LIKE ARRANGEMENT OF FINE FIBRILS

alveolar masses and cordons, for the most part just beneath the epidermis, as pointed out by Masson who believes that these bodies are comparable to the Wagner-Meissner tactile corpuscles. Still deeper in the dermis but directly continuous with the cord-like structures containing the fibrillary bodies, are elongated bundles which Masson believes to be nonmyelinated nerves. Scattered throughout the nevus, but sparsely in the undifferen-

tiated cellular area is a very fine fibrillated structure which has the appearance of being the cytoplasm of nevus nuclei, with which it is in close contact. In the cord-like structures of another pigmented mole this tissue is very abundant (fig. 4). It is not connective tissue of mesoblastic origin but appears to be peculiar



FIG. 4. CORD-LIKE STRUCTURES IN A MOLE

Note the fine fibrillated tissue that resembles neuroglia, tissue of Schwann or the fine texture nervous tissue of a neurofibroma.

to the nervous system in that it resembles neuroglia, tissue of Schwann or the fine texture nervous element of a neurofibroma.

Various differential staining methods have been applied to the foregoing moles, with the idea of obtaining a specific reaction for nervous tissue. However, further proof of their nervous nature has not advanced beyond that already learned with the use of

hematoxylin and eosin. With the latter stains, the undifferentiated nevus cells present the same staining qualities as the regenerative protective epithelial cells. The fibrillated bodies and the fine texture fibrillary tissue, stain a deep orchid in sharp contrast to the pink of the true connective tissue fibers. With Van Gieson and the Castroviejo modification of the Cajal trichrome connective tissue stain, the nuclei of the undifferentiated nevus cells stain a Vandyke brown with the former stain and a purple with

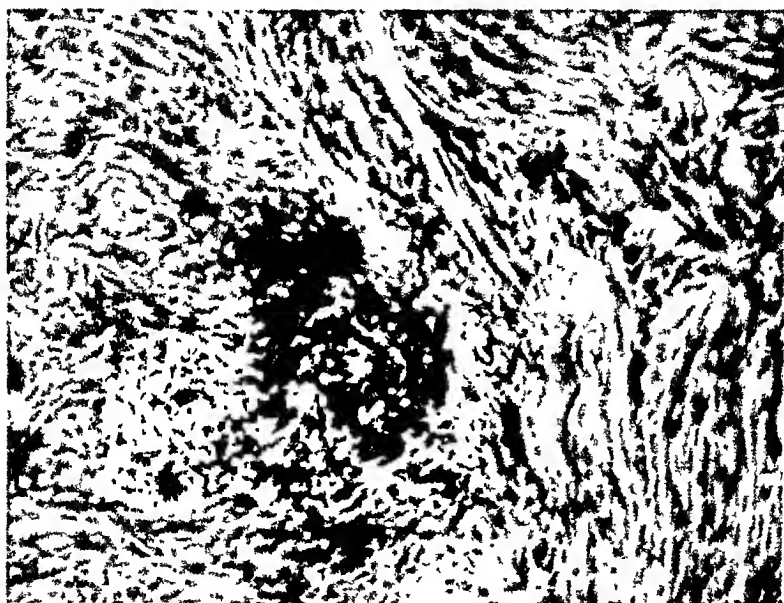


FIG. 5. PIGMENTED LESION OF THE SKIN OF THE DORSUM OF THE FOOT

This shows a picture typical of the blue nevus of Jadassohn in the left half and an intimately connected myelinated nerve in the right half.

the latter. The cytoplasm of these cells stains a pale yellowish green with both stains similar to that of the cytoplasm of regenerative protective epithelial cells. The fibrillary elements also stain a pale yellowish green with both stains in sharp contrast to the connective tissue which with Van Gieson stain is a light red, and with the modified Cajal stain there is a variation from pale blue to a bright green. These moles were stained with various silver and gold impregnation methods such as Orlandi, Cajal

and Bielschowsky, in an effort to bring out the nervous nature of the fibrillary elements. Mallory phosphotungstic acid stain and toluidin blue for neuroglia fibers were also used without avail. Recent investigations of Foot,^{10,11} by silver impregnation methods have corroborated the findings of Masson.

In the recent examination of a pigmented lesion of the skin on the dorsum of the foot we noted areas typical of the blue nevus of Jadassohn, one area of which contained undoubted nerve tissue

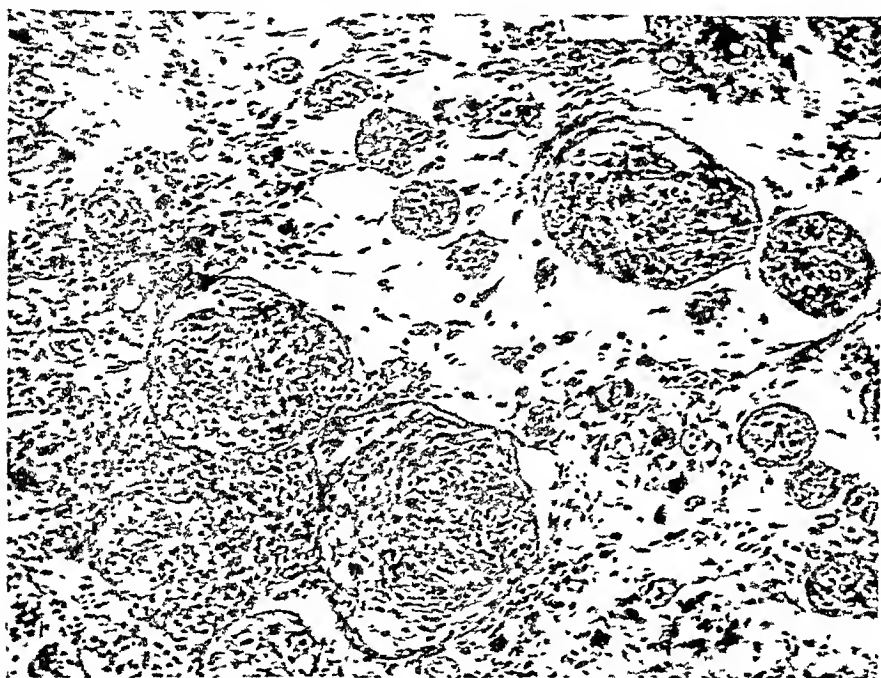


FIG. 6. ANOTHER AREA IN THE PIGMENTED LESION SHOWN IN FIGURE 5
Alveolar formations of typical nevus cells may be noted

that intermingled with the pigmented nevus elements (fig. 5). Other areas of this lesion disclosed alveolar formations of typical nevus cells (fig. 6).

In conclusion it may be stated that we agree with observers who hold that pigmented and nonpigmented moles or nevi and their malignant congeners the melanotic and amelanotic carcinomas or so-called melanomas are not of mesodermal origin nor are they of a connective tissue nature. We are also of the opinion that the so-called blue nevus of Jadassohn is probably not of mesodermal

origin but that it belongs to the same family as other pigmented nevi. It differs from the usual pigmented nevus in that its cells show a marked tendency to assume a spindle form thereby giving it a connective tissue effect. We agree with Unna and others who hold that moles are primarily of epidermal origin. We also agree with Masson to the extent that some moles show nervous elements; however, we are not in a position to agree with his original and ingenious hypothesis, that these nervous elements originate from the tactile nerves, neither can we agree that moles are of necessity *neuronevi*. It seems reasonable to assume that since the nervous system and the epidermis develop from the ectoderm, and since moles are of epidermal origin, that their spheroidal cells which we believe to be in an undifferentiated or embryonic state are atavistic to the extent that they are capable of differentiating into nervous tissue, and therefore in such light may be considered of a neuroepithelial nature.

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A FLASK FOR DILUTING ANTIGENS USED IN SERUM-TESTS FOR SYPHILIS

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The need for a simple, accurate method of diluting an alcoholic solution of lipoids with a salt solution, for use as antigen in serologic tests for syphilis, has been recognized for many years. The importance of a proper method of dilution lies in the fact that any given amount of antigen, if too slowly diluted with the correct amount of salt solution, will produce positive reactions with the serums of certain non-syphilitic persons. If too rapidly diluted with the same amount of salt solution it will produce negative reactions with the serums in certain cases of syphilis. This phenomenon is especially troublesome in the more sensitive flocculation reactions of which the Hinton test is an example.

I have had an excellent opportunity to observe the effect of variations in the rate of dilution of indicators (antigens) used in the Hinton test¹ in three laboratories under my direction. In each laboratory the method of executing the test and of mixing the reagents was based on the same instructions and the results, therefore, should have been nearly identical. Nevertheless, the indicators in each of the three laboratories differed from one another in gross appearance and the sensitiveness of the tests in the respective laboratories varied correspondingly. The differences in the indicators prepared in these laboratories grew out of failure to dilute all indicators in precisely the same way. One of the reasons for this can be readily seen if one considers that when salt solution is added, by means of a pipette, to an alcoholic solution of antigen, the rate of dilution will vary with the amount of indicator prepared at one time. For example, if one uses 1 cc. of the extract on a given day, and adds to it 0.8 cc. of 5 per cent salt solution,

drop by drop (a drop containing approximately 0.05 cc.), the ratio of dilution starts at 1 to 20; if on the other hand, 5 cc. of the alcoholic extract is required for a given day's tests, and one adds 4 cc. of 5 per cent salt solution, with the same pipette, drop by drop, the ratio of dilution begins at 1 to 100, or five times as great as when only 1 cc. of the extract is employed. The inadequacy

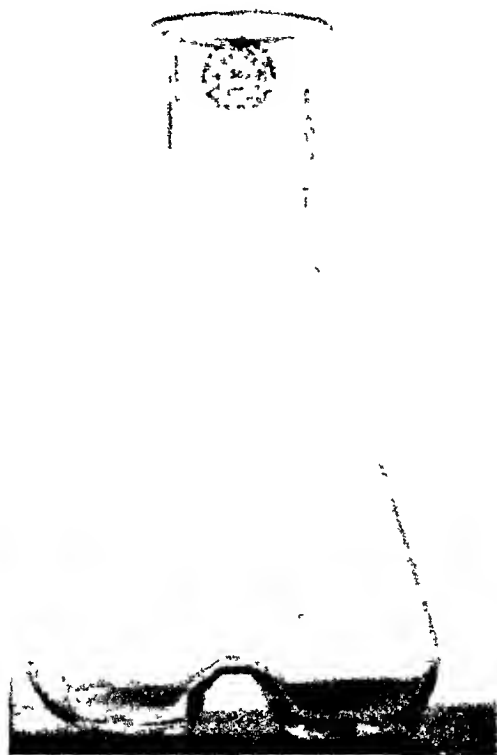


FIG. 1. INVERTED V-SHAPED PARTITION ON BOTTOM OF ORDINARY ERLIENMEYER FLASK

of the drop-method is further accentuated by the fact that a given technician can not at all times equally space the intervals between the drops. Furthermore, each technician will develop a speed of dilution that is individual, in spite of the most explicit directions. Because of these factors, the rate of admixing by any drop-method will vary and will correspondingly change the turbid-

ity and opalescence of the indicator, and, likewise, will affect the sensitiveness of this reagent.

In order to overcome these difficulties, I have recently modified the shape of an ordinary Erlenmeyer flask (fig. 1) by having an inverted V-shaped partition blown in its bottom. By this V-shaped partition two semicircular compartments are produced, each of which should hold from 3 to 5 cc. in flasks of 25 to 150 cc. capacity, while in the larger flasks (150 to 500 cc.) each of the two compartments should hold from 8 to 10 cc. For the preparation of 30 cc. of glycerinated Hinton indicator, a 125 cc. flask so modified is of suitable size, while a 500 cc. flask of the same description is suitable for the preparation of 300 cc.* Flasks of this type are not listed in any of the catalogs. I have had my flasks especially modified for this purpose by Macalaster Bicknell Company, of Cambridge, Massachusetts.

When a Hinton indicator is to be prepared in this flask, the alcoholic extract should be pipetted with great care into only one of the semicircular compartments; and the proper amount of salt solution into only the other. The admixture should be made by shaking the flask quickly from side to side so that the lipoids pass rapidly from one compartment to the other. This will effect a complete mixture almost instantaneously. By the use of this flask 30, 60, 90, 120, and up to 210 cc. of glycerinated heart indicator have been prepared repeatedly, each mixture possessing the same turbidity and opalescence and likewise giving the same reactions with the same serums. Controls, consisting of separate preparations of 30, then 90, and finally 210 cc. of the same indicator, carefully mixed in ordinary Erlenmeyer flasks, showed slight but definite differences in gross appearance. The use of the modified flask for diluting Hinton indicator has simplified the technique of this test. Since indicators of almost uniform sensitiveness can now be prepared by this method of dilution, the necessity of testing simultaneously with muscle extract has been eliminated from the Hinton test. Furthermore, the suspensoid obtained by dilution in this way has made it possible to

* For the preparation of antigen for the Kahn test, we have used a 25 cc. flask modified in the same way.

consider the slightest granularity as a precipitate, which signifies a positive reaction in the Hinton test.

Other simplifications of the Hinton test which have been made possible by the use of the modified flask are described in the third modification of the test.²

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THE LABORATORY DIAGNOSIS OF TUBERCULOUS MENINGITIS*

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The differential diagnosis of tuberculous meningitis from suppurative meningitis, poliomyelitis in the preparalytic stage, encephalitis, brain abscess or subdural abscess, and syphilitic meningitis often rests on the examination of the cerebrospinal fluid. The suppurative meningitides, as a rule, can be ruled out because of the high cell count, chiefly polymorphonuclears, and by finding the causative organisms in smears or cultures. Tuberculous fluids with high counts practically always contain large numbers of easily demonstrated bacilli, provided the observer takes the time to stain for tubercle bacilli when no other organisms are found in smears stained by other stains. The Wassermann test should rule out syphilis unless complicated by one of the other diseases. The other conditions are more difficult, since on gross examination the fluids show little differences. The demonstration of tubercle bacilli in smears from tuberculous fluids according to Hemenway⁵ should be possible in practically 100 per cent of cases. However, these excellent results are not duplicated in many laboratories, perhaps largely due to insufficient time given to the search.

In cases in which no bacilli are found the variations in the cell counts, globulin, gold curve, et cetera are not sufficient in themselves to differentiate between the various diseases mentioned above. Considerable aid, however, can be obtained by determining the glucose and chloride content. Giordano⁴ reviewed the pertinent literature fairly completely on the question of glucose, and stressed the fact that tuberculous fluids showed a marked

* Read before the Eleventh Annual Convention of the American Society of Clinical Pathologists, New Orleans, Louisiana, May 6-9, 1932.

reduction in sugar content, averaging 31 mgm. as compared with a normal average of 73 mgm. per 100 cc. of fluid. Fremont-Smith³, Stowe¹⁰, Kolmer⁶ and Boyd¹ reported the same findings and the latter pointed out that in poliomyelitis, encephalitis and brain abscess there was no decrease in sugar.

The value of chloride determinations has not been stressed sufficiently in recent textbooks on medicine and laboratory technique. Kolmer, however, stated that tuberculous fluids show a distinct decrease in chlorides, 500 to 700 mgm., as compared with normal values of 720 to 750 mgm. per 100 cc. Neal and Esslemont⁸, Pitfield², Levinson⁷, Fowweather⁵, Boyd and Fremont-Smith agreed with this finding and the latter two authors stated there was no such decrease in poliomyelitis, encephalitis and brain abscess.

This study is based on fluids obtained chiefly at the Buffalo City Hospital with a smaller number from the Buffalo General Hospital and the Pasadena Hospital. There were 147 fluids from eighty-seven cases of tuberculous meningitis, nineteen fluids from sixteen cases of poliomyelitis, seventeen fluids from eight cases of encephalitis, and thirteen fluids from eleven selected cases of severe acute meningococcal syphilis. All the fluids reported as tuberculous were proved by either smear, guinea pig inoculation or autopsy. All cases proved fatal within nineteen days after onset of the first symptoms referable to the nervous system. Most of the poliomyelitis cases were severe. Seven out of sixteen died of ascending paralysis. The encephalitis patients, eight in all, lived. These included both idiopathic lethargia cases and a few cases following respiratory infections. The eleven syphilis cases included are selected cases of active syphilitic meningitis showing marked symptoms. Until lumbar puncture was done and the patients studied carefully, the type of meningitis was in doubt. In all these the Wassermann reaction was positive. Three died and were proved at autopsy, and eight recovered under specific therapy.

All of the fluids, whether tuberculous or not, showed a slight frosted-glass appearance when the cells were slightly increased, and were definitely opalescent when the cells were increased to 500

or more per cubic millimeter. The teaching that the fluids in the above conditions are clear is far from the truth if the fluids are examined in the proper light against a dark background.

On standing overnight, or even for shorter periods in the case of fluids with higher cell counts, web formation was practically constant in the tuberculous fluids. Only a few with very low counts failed to show this characteristic phenomenon. Most of the fluids in the other conditions listed showed some fibrin formation, but the typical pine tree web of tuberculosis was only rarely seen in other conditions.

TABLE 1
CELL COUNTS IN SPINAL FLUIDS
(Column headings denote cell counts per c.mm.)

| TYPE | NUMBER FLUIDS | 0-50 | 51-100 | 101-150 | 151-200 | 201-250 | 251-300 | 301-400 | 401-500 | 501-700 | 701-1,000 | Above 1,000 | AVERAGE |
|--------------------|------------------|------|--------|---------|---------|---------|---------|---------|---------|---------|-----------|----------------|---------|
| Normal..... | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Tuberculosis..... | 116 | 14 | 15 | 18 | 23 | 9 | 13 | 10 | 7 | 3 | 1 | 3* | 209† |
| Poliomyelitis..... | 15 | 8 | 2 | 2 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1** | 79 |
| Encephalitis..... | 16 | 4 | 5 | 3 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 1** | 144† |
| Syphilis..... | 13 | 2 | 3 | 1 | 0 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 313 |

* Intraspinal serum in two cases.

† Serum treated cases excluded.

** Intraspinal serum.

Increased globulin as measured by the Pandy phenol and Ross-Jones ammonium sulphate methods and recorded as from 1+ to 4+ was noted in all the tuberculous and syphilitic fluids, averaging 2+ to 3+, whereas in the poliomyelitis and encephalitis fluid with corresponding cell counts the globulin averaged 1+ with many showing only a trace or heavy trace.

The results of the cell counts can best be seen in table 1. In one case of tuberculosis a cell count of three, and four days later of ten cells was observed. The results of guinea pig inoculation, decrease of sugar and chlorides and also autopsy findings proved the diagnosis correct. The average count in the whole tubercu-

losis series, exclusive of two counts over 1000 due to the irritation produced by administration of antimeningococcus serum before admission to the hospital, was 209 cells compared with seventy-nine in the poliomyelitis fluids and 144 for the encephalitis patients. Four fluids of the tuberculosis cases had counts from 500 to 1000 and one case showed 1120 cells. In these fluids the tubercle bacilli were abundant, and the cells were largely polymorphonuclears. The high counts in the syphilis fluids, including one of the 1109 cells in a patient who recovered, represented an extreme cellular response to a very active infection, and were in harmony with the clinical picture of severe meningitis shown by these patients.

TABLE 2
POLYMPHONUCLEARS IN SPINAL FLUID
(Column headings denote number of cells per c.mm.)

| TYPE | NUM- BER FLUIDS | 0-10 | 11- 20 | 21- 30 | 31- 40 | 41- 50 | 51- 60 | 61- 70 | 71- 80 | 81- 90 | 91- 100 | AVER- AGE |
|-----------------------|-----------------------|------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|------------|--------------|
| Normal | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tuberculosis | 130 | 69 | 6 | 6 | 11 | 12 | 9 | 2 | 6 | 9* | 0 | 24 |
| Poliomyelitis | 17 | 13 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1* | 0 | 17 |
| Encephalitis | 15 | 9 | 0 | 0 | 1 | 2 | 0 | 0 | 1* | 2* | 0 | 10 |
| Syphilis | 11 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 18 |

* Under tuberculosis, 2 cases had intraspinal serum (meningococcus). Under poliomyelitis and encephalitis, intraspinal serum.

Differential counts, table 2, showed on the average a high polymorphonuclear count in the fluids showing high total counts. In all types the lymphocyte was the usually predominant cell, but no matter what the infective agent, if the infection was hyperacute, polymorphonuclears were increased. Seven fluids from patients with tuberculosis, not given any serum intraspinally, showed 90 per cent of the cells to be polymorphonuclears, and twenty-four out of 130 fluids showed 50 per cent or more polymorphonuclears. On the average the poliomyelitis and encephalitis fluids showed less, about two-thirds showing less than 10 per cent. In two syphilitic fluids from a colored patient, in which the total cell counts were 450 and 144 respectively, 90 per cent

polymorphonuclears were found. A 4+ Wassermann reaction and autopsy revealed the syphilitic nature of the meningitis.

Tubercle bacilli were demonstrated in smears in forty out of fifty cases (80 per cent), and fifty-nine out of eighty-five fluids after November 1927, when the examination was made by one of us (A. G. F.) and not left to routine laboratory procedure which gave eighteen out of thirty-seven cases positive from 1924 to 1927. (see table 3). The best smears were obtained from fresh fluids before web formation had begun. The fluids were all thoroughly centrifuged twenty to thirty minutes or more, and supernatant fluids poured off completely and the tubes never allowed to be

TABLE 3
TUBERCLE BACILLI IN SPINAL FLUIDS

| | NUM- BER CASES | NUM- BER FLUIDS | POSITIVE SMEAR | | NEGATIVE SMEAR | | UNSATIS- FACTORY FLUIDS |
|--------------------------|----------------------|-----------------------|-------------------|--------|-------------------|--------|-------------------------------|
| | | | Cases | Fluids | Cases | Fluids | |
| 1924-Nov. 1927..... | 37 | 58 | 18 | 23 | 19 | 34 | 1 |
| Nov. 1927-Jan. 1932..... | 50 | 85 | 40 | 59 | 10 | 21 | 5 |
| Total..... | 87 | 143 | 58 | 82 | 29 | 55 | 6 |
| Children..... | 36 | 61 | 23 | 39 | 13 | 22 | 0 |
| Adults..... | 51 | 82 | 35 | 43 | 16 | 33 | 6 |

White 63. Black 21. Japanese 1. Indian 2.

tipped back past the horizontal in order to keep the sediment as concentrated as possible. The scummy sediments were picked up by means of capillary pipettes and smeared on warm slides and then stained by the Ziehl-Neelsen method, using a pale blue counterstain or occasionally picric acid. When web formation was present the web was teased onto a slide and spread as evenly as possible and to the same slide centrifugate of the rest of the fluid was added as above. We found the method of sedimentation depending on alcoholic precipitation of proteins not as satisfactory as the above methods. The alcohol method requires more fluid, the supernatant fluid is rendered useless for chemical tests, and the fluid is rendered sterile so that the material is lost

in case guinea-pig inoculation is to be resorted to. We were unable to obtain 100 per cent of positive tubercle bacilli findings as reported by some, for example Hemenway, but possibly our results would have been better had we had sufficient time to study smears over thirty minutes each, which must be done to give such perfect results. We did not test thoroughly the reputed advantage of examining the last bit of fluid drawn when enough fluid is removed to reduce spinal pressure to normal, but the idea seems quite plausible.

Cultures of small amounts of sediment of fluids were occasionally made on Corper's potato cylinder medium. Positive results were obtained with twelve out of fourteen fluids in which bacilli were found, and six out of eighteen in which none were found. When liberal amounts of sediment were on hand, 100 per cent positive cultures were obtained. Nine cultures were typed by Dr. Esmond Long and all found to be human strains.

The form of the colloidal gold curve was practically the same in the vast majority of the tuberculous fluids, the peak of the curve being about the 6th or 7th tube, the average reading being about 0001233210. No constancy was found in the poliomyelitis or encephalitis fluids. A few showed the same type of curve as the tuberculous, while others showed most precipitation in the luetic zone. The same occurred in the syphilitic fluids, the particular group of acute cases studied showing vastly different gold curves, varying from the usual curve seen in the average meningo-vascular neurosyphilitic.

Striking differential diagnostic evidence was obtained by quantitative determination of sugar and chlorides. These were made on fresh fluids only, by the techniques used at the time for blood chemistry, Meyer's modification of the Lewis-Benedict picric acid method for sugar and Van Slyke and Donleavy or Whitehorn method for chlorides. The fluids used as "normal" controls were obtained from consecutive fluids of ambulant patients tapped at various hours of the day, chiefly syphilitic patients given routine lumbar punctures following two courses of salvarsan. The effect of slight increase of sugar following meals may explain a few of the high sugar readings. None of these fluids showed any

increase of cells or globulin, change in the gold curve or serologic evidence of any nervous system involvement.

Our results in the various diseases (table 4) under discussion were similar to those of others, especially Giordano and Boyd. Tuberculous fluids practically uniformly showed a moderate to marked reduction in sugar. The average value was 36 mgm. per 100 cc., compared with the normal of 73 mgm., 84 per cent of the readings being below 45 mgm. All four cases showing sugar above 66 mgm. had been given intravenous glucose. No attempt was made to correlate the duration of the disease with the glucose concentration. However, in most cases in which repeated

TABLE 4
SUGAR CONTENT OF SPINAL FLUIDS
(Column headings denote mg. per 100 cc.)

| TYPE | NUMBER FLUIDS | 15-25 | 26-30 | 31-35 | 36-40 | 41-45 | 46-50 | 51-55 | 56-60 | 61-65 | 66-70 | 71-75 | 76-80 | 81-90 | 91-100 | OVER 100 | AVERAGE |
|-------------------|------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|----------|---------|
| Normal... | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 4 | 3 | 4 | 7 | 2 | 6 | 5 | 2 | 73 |
| Tuberculosis.... | 84 | 19 | 9 | 13 | 14 | 16 | 2 | 3 | 3 | 1 | 2* | 0 | 0 | 2* | 0 | 0 | 36** |
| Poliomyelitis.... | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 2 | 2 | 0 | 3 | 1 | 1 | 75 |
| Encephalitis.... | 15 | 0 | 0 | 0 | 1 | 0 | 1 | 2 | 0 | 1 | 0 | 0 | 1 | 5 | 3 | 1 | 76 |
| Syphilis..... | 12 | 0 | 0 | 1 | 0 | 0 | 0 | 3 | 2 | 1 | 1 | 0 | 2 | 2 | 0 | 0 | 63 |

* Intravenous glucose.

** Excluding cases given intravenous glucose.

determinations were done there was a slight to moderate drop in the sugar values as the disease progressed. Also on the average the fluids with the highest cell counts showed the lowest sugar content. The fluids from the poliomyelitis and encephalitis cases showed practically normal average sugar content, although some were decreased slightly, while others showed an increase. One patient with encephalitis who recovered showed 40 mgm. sugar and four days later 48 mgm. and a slight decrease in chlorides, 640 mgm. This is the only patient in the entire group or in our entire experience showing a decrease in both sugar and chlorides who did not have a tuberculous meningitis. One specimen in a case of syphilis showed a sugar of 35 mg. The

cell count in this patient was 742 and the chloride was normal. The other specimens from patients with syphilis averaged only slightly less than normal sugar.

Twenty-four normal fluids were studied fresh and then nineteen to twenty-four hours later without any preservative added. The average drop in sugar content was 4 mgm. per 100 cc. Three fluids showed a decrease of 10 mgm. and none more. Consequently we feel that sugar estimates on fluids drawn during the night are of some value if done during the following day, although all the figures in the above tables were obtained on fresh fluids.

Our experience with chloride determinations are practically in accord with other workers, especially Boyd, who stated that he

TABLE 5
CHLORIDE CONTENT OF SPINAL FLUIDS
(Column headings denote mg. per 100 cc.)

| TYPE | NUMBER OF FLUIDS | BELOW 500 | 501-550 | 551-600 | 601-650 | 651-700 | 701-750 | 751-1,000 | AVERAGE |
|--------------------|------------------|-----------|---------|---------|---------|---------|---------|-----------|---------|
| Normal..... | 31 | 0 | 0 | 0 | 1 | 9 | 20 | 1 | 708 |
| Tuberculosis..... | 65 | 0 | 10 | 21 | 27 | 6 | 1 | 0 | 603 |
| Poliomyelitis..... | 8 | 0 | 0 | 0 | 1 | 3 | 3 | 1 | 718 |
| Encephalitis..... | 12 | 0 | 0 | 0 | 2 | 4 | 5 | 1 | 700 |
| Syphilis..... | 9 | 0 | 0 | 0 | 0 | 6 | 2 | 1 | 697 |

had never seen a reading as low as 600 mgm. per 100 cc. in any other condition than tuberculosis, and that he has very seldom seen a case of tuberculous meningitis with a chloride reading of over 650 mgm. Fifty-eight out of sixty-five tuberculous fluids (table 5) showed values below 650 mgm. The fluids from cases of encephalitis averaged about normal, those in poliomyelitis slightly above, and those in syphilis slightly less, but all of these fluids showed chloride contents within the range shown by normal fluids.

From the clinical standpoint it is interesting that practically all the patients in the tuberculosis group who were in the hospital long enough for study showed evidence by clinical, x-ray, and postmortem findings of generalized miliary tuberculosis. Fifty-

one patients were adults, largely derived from the tuberculosis division of 200 beds in the Buffalo City Hospital. Of these the primary source of the infection in thirty-five cases was in the lungs, six in bones, five in hilum nodes, and one case each in the kidney, tubes and mesenteric nodes. Four patients were less than six months old, and twenty-one patients were under four years of age.

SUMMARY AND CONCLUSIONS

In the diagnosis of tuberculous meningitis from cerebrospinal fluid, the findings of tubercle bacilli on smear or culture or the production of tuberculosis in a guinea pig injected with the fluid is conclusive evidence of the disease. However, by the last two means the diagnosis can rarely, if ever, be made before the patient is dead. Smear examination if done on repeated samples should reveal bacilli in 80 to 100 per cent, depending on the technique and persistence of the examiner. But in the absence of a positive smear the following findings, especially when they are all present, (and they usually are) should warrant the tentative diagnosis of tuberculous meningitis: (1) Increased cell count, averaging about 200, producing a slight ground glass appearance in the fluid; (2) Differential count usually shows marked preponderance of lymphocytes but may show a moderate number of polys in smear-negative cases; (3) strong tests for globulin (2+ to 4+); (4) the formation of an inverted pine tree web on standing for several hours or overnight; (5) colloidal gold curve showing maximum precipitation in the 6th or 7th tube; (6) the sugar content moderately or markedly reduced, averaging about 36 mgm. per 100 cc. fluid, and (7) a decrease in chlorides below 650 mgm. Especially should the association of a marked decrease in sugar accompanied with a noteworthy decrease in chlorides in fluids not showing characteristics of a suppurative meningitis be stressed as a strong presumptive sign of tuberculous meningitis.

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THE ROSE BENGAL TEST OF LIVER FUNCTION*

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The common criticism of liver-function tests is that the liver is an organ of such protean activity and of such varied function that any single test fails to measure its true efficiency. This argument is not totally valid. The histological similarity of all liver cells is so complete that it is evident, more than in any other organ, that each cell carries on every function of the organ as a whole. Any functional test that will give a definite idea of the relative number of liver cells occupied at any one function, will measure quite accurately the capacity of the liver in general.

The following types of tests have been suggested for use:

(a) Nitrogen partition methods are cumbersome and have given inconstant results.

(b) Sugar metabolism tests have proved uncertain and contradictory. The best is probably the galactose excretion test of Bauer. Apparently this has been significant in acute degenerations only. So many endocrine and general metabolic factors enter into the sugar tests as to make the liver's part of the process difficult to evaluate. Quantitative determinations of blood sugar at frequent intervals in sugar tolerance tests, as recently outlined by Kerr et al.,⁶ may give a much clearer insight into certain liver functions. Unfortunately their detailed blood chemical analyses are impractical for general clinical use.

(c) The icteric index determination and Van den Bergh reaction are merely tests of the bile content of the blood and cannot be classed even as liver function tests. Their greatest value is in following the development or recession of jaundice.

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(d) The cinchophen oxidation test, recently presented by Lichtman,⁷ is interesting, but by his own estimation shares with the galactose test a lack of sensitiveness in chronic conditions of liver damage, such as the cirrhoses. Giving cinchophen to patients with damaged livers is obviously of questionable safety.

(e) The specific dye excretion tests, employing phenoltetrachlorthalein, brom sulphthalein or rose bengal, offer, to date, the most clear cut results. We have chosen to work with rose bengal because, as previously determined by Delprat,² it fulfills all the requirements of being a highly colored, non-toxic, crystalloid dye, specifically removed from the circulation by the liver. Brom sulphthalein might quite possibly be used equally well by our same technique, were the normal removal rate determined for a comparison.

We believe the technique of Rosenthal to be essentially faulty in assuming that a dosage of a 2 mgm. per kilogram of body weight will give a uniformly colored blood plasma that can be duplicated by a fixed artificial standard prepared in vitro. Obviously the blood volume per kilogram body weight varies tremendously between an obese woman of adiposogenital dystrophy type with small heart and vessels, and a lean, muscular man of less weight but larger vascular bed; and the color imparted to the blood by a fixed 2 mgm. per kilogram dose of dye must vary as much.

Our technique⁴ has been published in detail recently. Briefly it consists of injecting into an arm vein, irrespective of body weight, from 5 cc. to 10 cc. of 1 per cent or 2 per cent rose bengal in saline. Between these limits, at least, the amount injected does not matter. We have duplicated results on successive days, on the same patients, with the upper and lower limits of this dosage. The higher dosage gives more easily read solutions.

Two minutes from the start of injection the "standard" sample of blood is withdrawn into a clean, dry syringe and exalated. This is an in vivo mixture of the dye with the patient's blood, automatically corrected for pigment discoloration and for blood volume. Exactly six minutes later, the second, or "unknown," blood sample is withdrawn. The only exact point in technique

required is the timing of this six minute period. Many normal control tests have determined that the fall in color in that six minutes should be fifty per cent of the "standard." The plasma is separated from the two blood samples by centrifugation, cleared of turbidity and of most of its interfering pigment color by precipitation of proteins with two volumes of acetone and recentrifugation. The resultant dye solutions are crystal clear and easily compared in the colorimeter. We have been accustomed to remove about 8 cc. of blood for each sample, using 3 cc. of plasma and 6 cc. of acetone for precipitation. If the resultant dye solutions are still yellowish from bile pigment, it has been found of value to add three drops of saturated sodium hydrate solution to each, let stand about fifteen minutes in subdued light, and recentrifugate. Pure rose colored solutions usually result.

The calculation is simple if it is remembered that the unknown should give one half the colorimeter reading of the standard for one hundred per cent function. Higher readings mean correspondingly lower function. Algebraically expressed in simplest terms:

$$200 - \frac{200 R_s}{R_u} = \text{per cent of normal liver function}$$

where R_s is the colorimeter reading of the first (2 min.) sample and R_u of the second (8 min.) sample.

Because of the photosensitizing effect of rose bengal on the tissues, patients should be kept out of direct sunlight for a few hours after the dye is given. It is also advisable to warn them, and their nurses, that their stools will be red from the dye, lest they be alarmed at supposed intestinal hemorrhage.

Several chemically different dyes have been called rose bengal. It is essential to use an eight halogen atom dye as those with six halogen atoms are in part eliminated by the kidneys. We have used the "Special for Intravenous Use" rose bengal prepared by Coleman and Bell, chemically di-sodium-tetra-iodo-tetra-chlor-fluorescein.

RESULTS

Normal values, (85 to 110 per cent) 17 cases. (Normal pregnancy (3 cases); chronic colitis; cancer of breast with no metastases; peptic ulcer; asthma; influenzal pneumonia; chronic alcoholism (2 cases); chronic cholecystitis of mild grade (3 cases); trichiniasis; pre-eclampsia; intestinal obstruction; obstructive jaundice from scar tissue around duct (old operation). This last patient is of particular interest in showing 100 per cent liver function despite the presence of marked jaundice.

Gall-bladder disease. Rose bengal elimination was in inverse relationship to the acuteness of the disease. Two patients with mild chronic disease gave 100 per cent function. One patient with subacute disease without jaundice, 80 per cent, one patient with acute disease, unable to retain food for a week, 70 per cent. Two patients in acute attacks, with fever and leucocytosis, superimposed on years of previous attacks, gave 60 per cent function on admission and 70 and 80 per cent respectively after a few days of glucose medication and relief of symptoms. One patient with a history of two years of gall-bladder attacks, gall stones and a "hard liver" found at operation, showed 50 per cent function. One patient with complete obstruction from stone in the common duct gave 30 per cent function, rising to 100 per cent three weeks after passage of the stone spontaneously.

Toxemias of pregnancy. In three normal pregnancies the function varied from 85 to 100 per cent. One patient with pre-eclamptic toxemia of mild type showed 100 per cent, one patient with early pre-eclamptic toxemia, 80 per cent, which rose to 105 per cent in ten days after delivery. One eclamptic of severe grade showed 55 per cent function, rising to 85 per cent in ten days after cesarean. Two patients with hyperemesis gravidarum gave 55 per cent and 40 per cent of normal elimination of the dye, the former rising to 93 per cent on five days of intravenous glucose and relief of vomiting.

Cirrroses. Low values were uniformly obtained. The patients with typical Laennec type of four and five years duration gave 32 per cent and 33 per cent functions, one patient with Hanot type

showed 45 per cent, one following cinchophen hepatitis of seven months resolution, with recent disappearance of jaundice, 35 per cent function. Residual cirrhosis was doubtless present in this patient.

Alcoholism—chronic. Values here were 100, 90, 80 and 42 per cent. The last patient entered the hospital in his fourth attack of delirium tremens within two years. Alcoholic cirrhosis was evidenced by an enlarged liver and jaundice. A month later the rose bengal elimination had only increased to 48 per cent, while the jaundice was still present. This patient died about a month after the second test.

Carcinoma (secondary.) Three patients were studied. Two, with secondary cancer of the breast, had 78 and 80 per cent functions. The third, with liver metastases secondary to carcinoma of the gall-bladder, entered emaciated and starved, with 40 per cent function which rose to 70 per cent after five days of intensive glucose therapy by mouth and vein.

Diffuse hepatic damage. In addition to the patients with hyperemesis gravidarum mentioned, two are of interest. One with severe peritonitis following a ruptured appendix showed 60 per cent dye function. The other, entering with 67 per cent function and liver abscess of several months duration following peritonitis, had a slight rise to 70 per cent function following surgical drainage of the abscess, but failed to maintain clinical improvement. A month later, twenty-four hours before death, the rose bengal was 57 per cent. Post-mortem examination of the liver showed additional abscesses and severe damage, microscopically, to the liver cells between the abscessed areas.

These results accord quite closely with those previously reported,^{3,6,5} and we believe show very close correlation between the results of the test and the extent of the liver cell damage, numerically and structurally.

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THE PRESENT STATUS OF THE SEROLOGICAL DIAGNOSIS OF SYPHILIS, WITH SPECIAL REFERENCE TO BASIC PRINCIPLES*

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Additions to the overburdened literature on the serology of syphilis demand temerity and necessitate some attempt at justification. The present discussion arises from the belief that the renewed flood of contributions within recent years has tended to produce a rather paradoxical situation in that recent developments have clouded rather than clarified the serological study of syphilis which at present appears struggling between the Scylla of laboratory incertitude, on the one hand, and the Charybdis of clinical chaos, on the other. The impartial observer, especially if his angle of view be sufficiently comprehensive, cannot fail to find support for such a contention nor to appreciate also that the discussion has greatly narrowed in scope.

We do not appear to be concerned solely with syphilis as a diagnostic and therapeutic problem of paramount importance to patient, clinician, and community; nor primarily with the actual or relative value and utility of the various laboratory procedures available for its study. On the contrary, the discussion has had an insidious but perceptible drift into a much narrower circle and now appears to be transformed into a struggle for survival or annihilation, a series of individual combats, as it were, the purpose of which would seem to be the selection by elimination of a serological champion who, surrounded by prostrate foes, shall dominate the arena.

The contest now appears to have a double objective, based

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first, upon a clear-cut endeavor by the proponents of precipitation tests to eliminate the complement-fixation reaction from participation in the study of syphilis, and, second, upon what may soon assume the appearance of a squabble as to which precipitation reaction shall be the sole serological procedure to be used. This is stating the proposition with more bluntness than grace but that some such situation is at least in the making, if it does not already exist, can hardly be denied.

It seems, therefore, not only justifiable but profitable as well to regard the subject anew from a general rather than a particularized standpoint; not merely as proponents of this or opponents of that contention, but rather in an endeavor to see the problem as a whole with sufficient perspective to assign a true rather than an artificial importance to its many and varied phases.

This is the end, to be attempted if not achieved, of the present discussion in the pursuit of which it is profitable to reduce the subject, if possible, to basic, elemental principles, fundamental propositions, as it were, of paramount importance from which all else may be derived from logical processes.

THE FIRST PRINCIPLE

It has long been recognized that syphilis, in regard to both its recognition and control, presents one of the most intricate and puzzling problems in the entire field of medicine. Because of the tremendous potentialities involved and the far-reaching and sometimes disastrous possibilities of its aftermath, the diagnosis of syphilis is of paramount importance to the patient; for the same reason, so gravid with disaster is an erroneous diagnosis that every precaution should be evoked to prevent a missed diagnosis on the one hand as well as an erroneous assumption of the presence of this disease on the other.

It is essential, therefore, in any consideration of this infection, that the interests of the patient must be always predominant, and it may be set down as a first and highly important principle, that *the diagnosis of syphilis, however achieved, should be surrounded by every possible safeguard*, regardless of the time, the labor, the expense, or the minutia involved.

THE SECOND PRINCIPLE

One cannot consider the problem of syphilis, even in the most cursory manner, without realizing, that the recognition of any specific pathological entity as the underlying mechanism responsible for symptomatic manifestations is less often, and undoubtedly with less certainty, achieved by inspiration than by a thorough and painstaking consideration of all the possibilities which come within the knowledge and recollection of the observer. Nor can it be overlooked that the number and diversity of these possibilities is in direct proportion to his ability to detect in the patient all the manifestations of disease, no matter how cryptic or how minor, and to attempt their correlation with as many possibilities as the broadness of his information includes.

Particularly is this true and of essential importance in syphilis because of the ability of this disease to simulate others and to manifest itself, especially in its congenital, latent, concealed, and later stages, in cryptic and even bizarre fashions the true evaluation of which may well tax the skill and acumen of the clinician to the uttermost.

Upon these well-recognized but not always equally well-emphasized factors is based the maxim that it is well "to be quick to suspect syphilis but slow to diagnose it," which is only another way of emphasizing the many, diverse, and obscure ways in which the disease may first present itself to the physician.

It may well be accepted therefore as a second essential principle applicable to this tremendous problem, that *the diagnosis of syphilis should be based upon a careful, well-balanced consideration of all the data, however obtained, rather than predicated solely upon one or two isolated particularized facts or findings.*

THE THIRD PRINCIPLE

The deeper one delves into the study of disease in general and of syphilis in particular, the more obvious it becomes that in the detection and measurement of its manifestations a variety of methods must often be called into play, among them those embodied in the varied procedures of the clinical laboratory.

In few conditions is this more often of importance than in syphilis, for the accumulated experience of the past has demonstrated beyond cavil that the problem of syphilis may be and often is insolvable by all the usual avenues of clinical approach, no matter how well applied.

Past experience has demonstrated also beyond any need of further contention that of all the available avenues of laboratory approach to the study of syphilis, none are more generally applicable nor more delicate and reliable than the serological.

In view of these facts, then, as a third principle it may be stated that *a careful and intelligent study of syphilis is impossible without constant resource to laboratory avenues of investigation, especially serological studies.*

The proposition thus stated is now practically self-evident and has become well established through the numerous, varied, and cumulative investigations of innumerable workers and there is no dispute of the contention that, when wisely applied and intelligently interpreted, serological studies are of inestimable value in syphilis, just as they may be productive of incalculable harm unless properly safeguarded.

It is necessary that these elementary principles be reiterated and emphasized because an unfortunate tendency of modern times, arising from modern developments and refinements in the procedures of the laboratory, has been the development of an attitude which ascribes to laboratory procedures a paramount rather than an ancillary importance in the study of the patient.

In other words, many physicians have shown a tendency to make the laboratory investigations a prime rather than a complementary factor, and to think of them and utilize them as "tests" the results of which may be taken as indicating the presence or absence of disease. This is an erroneous as well as a dangerous conception, for the true function of laboratory procedures is simply and solely that of methods for the detection and measurements of response to varied stimuli, and as such they constitute simply one, though a very important and often indispensable, phase of the examination of the patient. It is only when thus regarded and so utilized that laboratory procedures can be wisely

and safely applied, and it is to this end that clinical pathologists have loudly decried undue and blind reliance upon them.

THE FOURTH PRINCIPLE

It is, therefore, both timely and essential, to accept as a fourth principle that *laboratory procedures must be regarded solely as constituting a single phase in the examination of the patient*; as furnishing means for the detection and measurement of the response to varied and not always pathological stimuli, recognizing that their real and greatest value rests upon their intelligent interpretation from which the nature and often the degree of stimulus responsible for their results may frequently be derived by inferential reduction.

To the cursory or superficial observer it would seem unnecessary to repeat and re-emphasize dicta apparently so trite as those embodied in the principles above stated. But the experience of serologists and syphilographers furnishes many and tragic examples of their neglect and leaves no room for doubt that many individuals are stigmatized as syphilitic and condemned to years of treatment and observation on the basis of what, after all, is really only a piece of paper inscribed with the symbol "plus four reaction," while in many others the spirochetes pursue the unmolested and even tenor of their malevolent way because a similar piece of paper bears the magic inscription "negative."

Serological methods constituted the first as well as one of the greatest advances made in the study of syphilis; but they cannot and must not be regarded as unimpeachable and infallible, nor can they escape compliance with that law of common sense which compels recognition of the fact that, in the last analysis, it is not the test but its interpretation which is of significance.

Accepting, then, serological procedures as indispensable in the study of syphilis, serologist, syphilographer, and clinician must next consider and establish, as definitely as possible, their relative value and reliability, and, what is of essential importance, must determine how their practical utilization may be best safeguarded against malapplication and malinterpretation.

It is obvious that so long as there are cryptic and latent stages

of syphilis in which no amount of clinical skill, experience, or acumen suffices to discover the presence of the disease nor to establish evidences of its eradication or control, serological procedures constitute a most valuable and important clinical weapon of attack.

It is equally obvious also, that the present tendency is to narrow the scope of serological investigations, and to select one method in preference to all others. To this proposition many appear to be more or less committed while others, approaching the other extreme, would combine the use of all available methods, and still others, seeking the middle path, would check one procedure by another varying in principle or technic.

While variations in technic are daily becoming more numerous—many, however, being but exceedingly minor deviations from established methods, so minor as scarcely to justify the naming of a “new” test, serological procedures in syphilis, whatever their nomenclature, fall into one of two groups and are based upon somewhat similar if not identical mechanisms.

On the one hand, are those dependent upon the ability of syphilitic serum to fix complement in the presence of a suitable antigen, and on the other, those arising from the property of syphilitic serum producing flocculation or precipitation of a suitable colloidal suspension.

The first of these, the complement-fixation reaction, is the older and has been subjected to the most prolonged and intensive investigation to date; the second, the precipitation test, is the newer and the one about which at present the most acute interest seems to center.

It is exceedingly unfortunate that the complement-fixation test is so commonly spoken of as the “Wassermann test,” for the procedure as conducted today bears no resemblance to that originally described by Wassermann and has in common with it only the basic principle underlying the mechanism upon which it depends.

It is still more unfortunate, and this indeed is the source and cause of much of the clinical confusion undoubtedly existing, that to far too many clinicians a Wassermann test is a Wasser-

mann test regardless of how or by whom performed, and the results of which are accepted or discarded with little or no endeavor to determine the reliability of the method used or to estimate the competence, skill, and conscientiousness of the worker by whom it was applied.

It is, without exaggeration, both terrible and inexcusable that diagnoses should be made or discarded and treatment begun or abandoned for no better reason than the existence of a report, which too often represents almost all the investigation made to determine the presence or absence of evidence of syphilitic infection, and concerning the reliability of which but little concern is manifested, regardless of whether the reaction be one of complement-fixation or precipitation.

THE FIFTH PRINCIPLE

It should be recorded as a fifth principle, therefore, that *a joint and interlocking responsibility rests upon both serologist and clinician entitling each to demand somewhat of the other and obligating both to a joint utilization of their combined resources in the interest of the patient.*

Of the serologist it may be expected that he be truly a serologist well-grounded in the elements of this specialty and not merely a manipulative expert; that he should not be unalterably wedded to a single method but eager and willing to investigate the merits of new proposals and, when warranted, to adopt them entire or in part, and finally, that, when called upon, he should have sufficient clinical experience to correlate correctly the combined clinical and laboratory data of a particular case.

Of the clinician it may be demanded that he be sufficiently acquainted with the rationale and mechanism of serological procedures to apply them intelligently and interpret them wisely; that he realize that a positive or negative serological report signifies solely that the blood examined did or did not contain at the time of examination complement-fixing or precipitating substances and that the significance of the reaction is a matter wholly beyond the fact of its occurrence, and finally, that if he assumes the responsibility for the interpretation, he also assume the

responsibility for acquiring sufficient familiarity with the details and factors influencing the result, that he be perfectly familiar with the limitations of the test in question, and competent to evaluate properly its results.

In view of what has already been outlined it is necessary now to consider the methods properly applicable to the serological study of syphilis and from the comprehensive, intensive, critical, and even hypercritical studies of over a quarter of a century the sixth principle is readily derived.

THE SIXTH PRINCIPLE

The complement-fixation reaction, in its modern, refined, and perfected form as exemplified, for instance, by the quantitative method devised by Kolmer, constitutes not only one of the most valuable and reliable laboratory procedures at present available, but also when positive, may be regarded with confidence as the most delicate and constant single sign of syphilis.

So extensive have been the investigations upon the results of which this principle is based, and so thoroughly have they been discussed and evaluated in the voluminous literature of this subject, that there is no need to reconsider them here.

So comprehensive, likewise, have been the studies of the quantitative method of Kolmer that its status is no longer a matter of contention: If one is not willing to accept it as the best at present available, it can be and is accepted as a standard with which other methods, precipitation as well as complement-fixation, may well be compared.

My own fairly extensive experience with it has satisfied me with its delicacy, reliability, relative specificity, and clinical applicability in every respect. *But it must be said that this applies only when the method is used as described without distortion of its principles or evasion or omission of its essential minutia.*

THE SEVENTH PRINCIPLE

Modern developments in the field of serology have furnished data from which a seventh principle may be derived, based upon the fact that syphilitic serum possesses a colloidal instability as

a result of which, when brought into contact with a suitable colloidal antigenic suspension under properly controlled conditions coherence or aggregation of suspended colloidal particles occurs.

So constant is this phenomenon and so extensively has its occurrence been studied that it may now be stated as a seventh principle: *that so great is the practical specificity of precipitation tests under properly controlled conditions, that they may be accepted as valuable additions to the serological study of syphilis, and as useful if not essential adjuncts to the complement-fixation test.*

The practical value of precipitation tests in the serological study of syphilis is now definitely established by the work of Kahn, Kline, Hinton and others who have subjected these and similar methods to long, careful, and extensive study, and, as just stated, precipitation reactions have now an established standing and their usefulness is no longer a matter of dispute.

What is a matter of contention, however, is the exact status to be accorded them; whether they shall be used as adjuncts to the time-tried complement-fixation test, or whether they shall constitute a sole and sufficient criterion for the laboratory study of syphilis. Before this latter assumption can be accepted with safety and without reserve many factors must be carefully considered, among the more important of which are these: It cannot be overlooked that, regrettable and, in fact, almost inexcusable as it may be, in no small proportion of cases the serological investigation for evidence of syphilis is all too often restricted to the examination of a single specimen.

Any serological procedure to be accepted as an ideal, infallible, and final procedure the results of which shall be accepted as incontrovertible, necessitates, under the circumstances, that the following criteria shall be fulfilled: (1) It must possess an absolute specificity for the disease. (2) It must infallibly and consistently detect the presence of syphilitic reagin in any and all stages of the disease. (3) It must not give a positive reaction in the absence of syphilitic reagin. (4) It must be, to all intents and purposes, "foolproof."

It is obvious, of course, that no single procedure exists which

can meet these requirements. Nor is it possible that any such procedure will ever be devised because of factors inherent in the disease and, what is of still greater importance, the reaction of the patient to the disease.

THE EIGHTH PRINCIPLE

Until the nature of syphilitic reagin is discovered it is impossible, to devise or elaborate truly biologically specific tests for its presence.

Rather paradoxically, however, the almost incalculable amount of work which has been done in this field has established beyond question what may be accepted as an eighth principle in the field of serology, namely: *that while both the complement-fixation and the precipitation reactions are biologically non-specific, they possess, nevertheless, an extraordinary degree of practical specificity when properly performed under carefully controlled technical conditions, so much so that positive reactions are consistently encountered in only one disease other than syphilis, namely, yaws.*

It must be emphasized, however, that this can be accepted as true only under acceptable and controlled technical conditions and only when these are strictly adhered to and competently carried out. The statement cannot be applied to nor accepted of any and all of the numerous and diversified methods collectively and generally spoken of as "Wassermann tests," nor of any and all of the precipitation methods. On the contrary, it may be said with little fear of error that it applies in the strictest sense to but relatively few complement-fixation methods, among them that of Kolmer, and to but few of the precipitation methods.

It should also be emphasized, however, that there is no method of conducting complement-fixation or precipitation tests at present available, nor can it be expected that any will ever be devised, with which it will be impossible to obtain non-specific false positive reactions. This is inescapably true because false positive reactions may be so readily produced by technical errors due to carelessness, inexperience, or manipulative ineptitude.

This fact alone renders blind reliance upon any single isolated serological reaction exceedingly dangerous unless it has been

carried out with a high degree of technical skill fortified by thorough training and supplemented by a conscientious sense of the responsibility involved.

In view of the influence of technic upon the production of non-specific reactions, it is of great practical importance to emphasize certain technical features of both complement-fixation and precipitation tests which are of importance in this connection.

1. Much has been said of the simplicity of the precipitation as compared to the complement-fixation tests; but equal emphasis has not been laid upon the fact that the underlying mechanism of both is quite similar if not identical, and that, to this extent, one is no simpler than the other. Nor has it always been equally emphasized that, as has been amply demonstrated by practical experience, it is far easier to train a technician to conduct a complement-fixation test with technical accuracy so that the results shall be clear-cut and without question, than to train a technician to perform precipitation tests with an equally consistent degree of skill.

It is my opinion that the inherent complexity of the complement-fixation test should be its greatest safeguard in that the necessity for careful, thorough, and extensive training fortified by experience required for its intelligent performance should restrict its performance to those so trained and adapted. For it may be stated without equivocation that neither complement-fixation nor precipitation tests should be performed by those whose knowledge of their minutia is, at best, superficial.

2. Regardless of the technical skill and care exhibited by the worker, the weakly positive, indefinite, "plus-minus" reaction will always be a source of trouble and incertitude largely because of the uncontrollable factor of the personal equation which influences their determination.

It is difficult to see how the reading, classification, or interpretation of such reactions can ever be safely standardized without loss in delicacy and specificity and to this extent precipitation tests present difficulties not encountered in the complement-fixation reaction, to which, in this sense, they are inferior.

3. Perhaps the one feature of precipitation tests which has

been most clamorously extolled is the rapidity with which they may be completed.

Rapidity, however, cannot be accepted as sufficient reason for the exclusive adoption of precipitation tests unless one accepts also the necessity for precipitate haste in diagnosis; and wherein lies the necessity for instantaneous diagnosis?

It cannot be because of danger to the patient by reason of delay of a few hours or even days, because syphilis, at best, is an intractable disease and its treatment slow and tedious. It is not a disease in which life, death, or ultimate cure is a matter of days or months; efficient treatment is a matter of years. In the early case, by the time a serological reaction is detectable the disease has been systemically disseminated; in the late case, or one rediscovered years after insufficient treatment, a further delay of forty-eight to seventy-two hours is of little importance.

There should be no opposition to nor regret for whatever may be necessary in time or labor to render the diagnosis of syphilis free from error or to safeguard the treatment of this dreaded malady.

As has already been said, the ideal serological test for syphilis should be capable of detecting the presence of syphilitic reagin in any and all stages of the disease, a requirement incapable of fulfillment by any method now available or, indeed, ever to be devised. The reason is obvious: Because the production of reagin is dependent upon and in great measure proportionate to the degree of interaction between the invading spirochetes and the reacting tissues. It is not enough that spirochetes be present. They must attack the tissues and the tissues in turn must react to the attack. Unless these postulates are fulfilled the production of reagin is impossible. Even the most elementary consideration of syphilis forces the realization that spirochetes may be present and viable and yet tissue reaction, if not absent, may be so minimal that reagin production will be far below the conceivable limits of detection.

It is this proportionate relation between tissue reaction and reagin production which explains the failure to obtain positive serological reactions immediately following the initial spirochetal

inyasion, during the early days following the appearance of the chancre, and in the later, latent, and inactive stages of the disease.

THE NINTH PRINCIPLE

This is also the underlying reason for what may be taken as the ninth principle applicable to the serological study of syphilis, namely, that *there is no serological procedure at present available, nor is it probable that one will ever be devised, with which a false negative reaction may not be obtained.*

Where the management of syphilis is in competent hands, false negative serological reactions are of relatively minor importance and productive of but little harm as they will be subjected to interpretation in the light of all the data pertaining to the particular case and checked by further and additional studies.

But as long as it remains true, as it is true in a definite proportion of cases, that the diagnosis of syphilis and, particularly, decisions concerning the necessity for initiation or cessation of treatment are dependent largely upon the results of infrequent and perhaps isolated serological examinations, the occurrence of false negative reactions will remain a matter of practical importance.

It must not be forgotten, also, that false negative reactions may arise from the spontaneous and sometimes marked fluctuation in reagin content which, for reasons as yet unknown, may be concomitant with any stage of the disease and any degree of activity.

This one phenomenon alone is sufficient illustration of the danger of relying upon an isolated negative reaction as indicative of the absence of syphilis or as furnishing a safe and infallible basis for the cessation of treatment.

There is still one other explanation for the occurrence of false negative reactions based upon the practical experience of serologists at large which collectively has demonstrated a selective affinity on the part of reagin for various antigens.

This is more commonly discussed in terms of antigen sensitivity and refers to the ability of antigenic suspensions to react with reagin.

That this is a peculiarity of the reagin, however, rather than of the antigenic colloidal suspension is shown by the fact that antigenic suspensions reacting with a majority of positive sera will fail to react with a particular positive serum, while the same serum will react strongly with another antigenic suspension of no better quality than the first.

While the occurrence of this phenomenon has been rendered comparatively negligible by present methods of antigen preparation giving products of great sensitivity, it has not been totally eliminated as a factor of some importance.

It is this phenomenon which is responsible in every series of parallel complement-fixation and precipitation tests, for the occurrence of a variable number of tests in which, with sera simultaneously examined, the reaction is in one case positive to the complement-fixation test and negative to the precipitation test, while in another the reverse is true. This in itself is not a disadvantage because one test may thus be used to check the other and the chance of a false negative reaction thereby reduced.

If this possibility furnishes an added reason for avoidance of undue reliance upon any single method, especially when it is to be applied to a single specimen, by the same token it may be taken as indicating very strongly the advisability of simultaneously utilizing both methods of procedure on every serum tested.

THE TENTH PRINCIPLE

So obvious does the wisdom if not, indeed, the necessity for such a course appear from the numerous and cumulative studies that have been made that it may be accepted as a tenth principle in the serological study of syphilis, that *the simultaneous use of both complement-fixation and precipitation tests on every serum should be routinely practiced* that one reaction may both complement and corroborate the other and the chance of both false positive and false negative reactions be thereby minimized.

We are considering now, not the question of diagnostic interpretation of the reaction, because, as has already been emphasized this is always to be based upon a consideration of all available data and not merely of the serological data alone; but simply

whether the reaction shall be reported to the clinician as positive or negative.

It is apparent, of course, that this must be governed in large measure by the serologist's experience with the methods in question and his balanced judgment as to their comparative reliability, delicacy, and specificity.

My own course in such case is governed by my own past experience as well as by the reported experience of others. My routine complement-fixation test is the six-tube quantitative method of Kolmer and my routine precipitation test is the slide precipitation method of Kline. Personal experience with Kolmer's method, begun before its publication and fortified by its consistent use and many and varied comparative trials during the succeeding ten years, has taught me to accept it without question as a reliable standard of comparison. I consider it to be probably and with very little question one of the most, if not the most, reliable and delicate methods available and I am convinced that with this method, when carefully and exactly applied, nonspecific reactions are encountered with extreme infrequency, if, indeed, they occur at all. It is not, however, free from the occurrence of false negative reactions for the reasons already outlined.

My experience with the Kline test began with its publication in 1926 and since then it has been used by me in many thousands of tests in series with simultaneously conducted Kolmer tests. I believe the Kline test to be the equal if not the superior of the Kahn test and to possess a high degree of specificity and delicacy. It also, and for the same reasons as given above, is not without false negative reactions and, what is of greater importance, it occasionally gives false positive reactions.

Like all precipitation tests it presents difficulty in reading and evaluation of border-line, weakly-reacting sera because of the impossibility of compensating for or eliminating the personal equation in reading such reactions. "Plus-minus" reactions, I believe, should be disregarded except, perhaps, when the test is used as a control of treatment. To this extent the test is less satisfactory than the Kolmer test.

For these reasons, when the Kolmer test is positive and the

Kline test negative, I refer now to at least a +3 reaction in the first tube of the Kolmer set-up—I report the reaction as positive.

When the Kolmer test is negative and the Kline test is positive I prefer to repeat the test either with the same serum, using another Kolmer antigen, or, preferably with another specimen. Because of the occasional discordance in the results of the two tests when simultaneously conducted it has been suggested that, perhaps, it would be wise to use a number of different methods, for instance, to use the Kline, the Kahn, the Hinton and the Kolmer tests on each serum. Such a proposal has various practical and important disadvantages.

In the first place, though the above list seems formidable enough it by no means exhausts the acceptable methods now available, some of which, at least, it would be difficult to exclude fairly or on logical grounds. This proposal in turn leads to the possibility that the withdrawal of the sample for testing might become a formidable operation followed by a period of convalescence and has in it the elements of a *reductio ad absurdum*.

For it is obvious that in the presence of discordant results some one or more reactions must be taken as the standard of comparison from which a conclusion can be drawn. Certainly, all the reactions cannot be taken as of equal value. If they were all would not be needed. If they are not, the most reliable should suffice.

If the interpretation of the reaction as positive or negative is to be made by the serologist the practical difficulty above outlined remains, but if the reactions encountered are reported to the clinician without summation, evaluation, or conclusions, the difficulty is enormously increased, so much so that the results of the whole procedure may be nullified and serological methods greatly discredited.

For it must be constantly remembered that the bulk of serological reports are made, not to syphilographers, but to clinicians at large many of whom are not syphilographers in any sense of the word and many of whom are far from skilled in the interpretation of serological reactions. No further proof of this is

needed than the inquiries, common in the experience of any serologist, as to the meaning and significance of the term "anticomplementary reaction."

What will happen then when four reactions show varying degrees of positivity and three are negative, and the patient is anxious to hear that he never had the disease or, if he had, that he has had enough treatment?

It seems to me far safer and more satisfactory for all concerned for the serologist and clinician to be well versed in the capabilities and limitations of one acceptable complement-fixation test and one acceptable precipitation test and to use them simultaneously, than to introduce unnecessary multiplications of unnecessary complications by a multiplicity of methods.

In the present state of our knowledge of the serological aspects of syphilis if it can be considered safe, wise or even justifiable to restrict arbitrarily or dogmatically the serological study of syphilis to any one single procedure, it may be demanded, at least, that any such postulate should be confronted with a full consideration of all the fundamental principles involved which, I believe, may thus be summarized:

SUMMARY

1. The diagnosis of syphilis, however achieved, should be surrounded by every possible safeguard regardless of the time, the labor, the expense, or the minutia involved.

2. The diagnosis of syphilis should be based upon a careful and well-balanced consideration of all the data, however obtained, rather than predicated upon one or two isolated or particularized facts or findings.

3. A careful and intelligent study of syphilis is impossible without constant resource to laboratory avenues of investigation and especially serological studies.

4. Laboratory procedures must be regarded solely as constituting a single phase in the examination of the patient; as furnishing means for the detection and measurement of the response to varied and not always pathological stimuli; recognizing that their real and greatest value rests upon their intelligent interpretation

from which the nature and often the degree of stimulus responsible for their results may frequently be derived by processes of inferential deduction.

5. A joint and interlocking responsibility rests upon both serologist and clinician entitling each to demand something of the other and obligating both to a joint utilization of their combined resources in the interests of the patient.

6. The complement-fixation reaction, in its modern, refined, and perfected form, constitutes, not only one of the most valuable and reliable laboratory procedures at present available, but also may be regarded with confidence, when positive, as the most delicate and constant, single symptom of syphilis.

7. So great is the practical specificity of precipitation tests, under properly controlled conditions, that they may be accepted as valuable additions to the serological study of syphilis and as useful, if not essential, adjuncts to the complement-fixation test.

8. While both the complement-fixation and precipitation reactions are biologically nonspecific, they possess, nevertheless, when properly performed under carefully controlled technical conditions, an extraordinary degree of practical specificity, so much so that positive reactions are consistently encountered in only one disease other than syphilis, namely, yaws.

9. There is no serological procedure at present available, nor is it probable that one will ever be devised, with which a false negative reaction may not be obtained.

10. The simultaneous use of both complement-fixation tests and precipitation tests should be routinely practiced on every serum that one reaction may complement and corroborate the other and the chance of both false positive and false negative reactions be thereby minimized.

A DISCUSSION OF THE PAPERS ON SERODIAGNOSIS OF SYPHILIS BY DOCTOR KILDUFFE AND DOCTOR LEVINE*

RUTH GILBERT. The aspects of the problems concerned with the serodiagnosis of syphilis which have been presented this afternoon seem especially worthy of consideration. Possibly in no other medical field is America taking

*See the JOURNAL, 2: 319, 1932.

the lead more definitely than in the serology of syphilis. I think that we in this country may feel justly proud of the achievements that have already been accomplished.

As was exemplified in the work presented today, the importance of delicately adjusted procedures which will give high percentages of reactions with specimens from syphilitic patients and a minimal number with those from persons without history or clinical evidence of the disease cannot be too greatly stressed. Experience with the work in a number of large laboratories in this country and more direct contact with that in New York State, leads me to believe that the chief problem at present is to secure sufficiently sensitive results. The tests widely used in the United States apparently tend to furnish very small percentages of unexplained reactions. I am sure that you have all observed that, barring those due to clerical or technical errors, the number of this type of reaction bears a distinct relation to the care, ability, and primary interest shown by the clinician in charge of the patient. With the skilled syphilologist, the number of apparently false reactions secured becomes practically nil, the majority of such results being found to occur with specimens submitted from institutions where the physicians are primarily interested in other fields of medicine. In this connection, the possibility of some drain on the vitality of the organism, such as that due to trauma, febrile or malignant disease or pregnancy, being a contributing factor in the activation of a latent, acquired, or congenital syphilis may sometimes be overlooked.

Dr. Levine and Dr. Kilduffie have stressed the importance of proper control of the work. The use of two different procedures, as recommended by them and at the League of Nations conferences, if the results are comparable, provides assurance of technical accuracy; or, if they differ, of the necessity for confirmation. The small percentage of sera which react with one procedure and not with another, when the methods approximate each other in sensitivity, certainly deserves further study.

Arrangements for sufficient standardization of complement-fixation tests and of precipitation tests also, at least to the extent that features generally recognized as desirable be incorporated in the procedures employed, would, I believe, tend markedly to improve the service in certain laboratories particularly some of those where the volume of the work done is not large. The establishment of means for providing reagents which could be used as standards should be especially helpful. Dr. Ruediger's suggestion relative to having available suitably preserved serums of designated titer, seems an important contribution.

It may not be amiss to reiterate the point made by Dr. Kilduffie that serological tests for syphilis should be conducted under the supervision of persons who are familiar with the symptomatology of the disease as well as with laboratory procedures. In the last analysis, the results of these, as of other diagnostic tests, must be interpreted in the light of clinical manifestations.

In view of what has already been accomplished, it is to be sincerely hoped

that adequate coöperation can be secured in perfecting and standardizing serological procedures so that every physician in this country who makes use of efficient laboratory service may feel assured that the most reliable data obtainable will be furnished him by his serologist.

B. S. KLINE. Personal experience with the Wassermann test by some half dozen technics in the past twenty-one years has revealed something of the temperamentality of the five ingredients, something of the mechanism of the reaction and something of the clinical application of the test. Seven years experience with various precipitation tests for syphilis has revealed something of the temperamentality of the two ingredients, something of the mechanism of the flocculation reaction and something of the clinical application of the tests.

The value of good Wassermann tests has been so amply demonstrated in the past twenty-five years that the state of flux mentioned by the Chairman has to do, I believe, with the value of the precipitation tests for syphilis. To what extent these tests will be developed and just what their ultimate clinical application will be is now a matter of opinion. At this time, I would like to discuss certain facts relating particularly to the precipitation tests.

In the first place, when some acetone insoluble fraction of alcoholic heart muscle extract either directly or from alcoholic solution is emulsified in a small quantity of water or salt solution, it is dispersed in the form of discrete globules averaging about 6 micra in diameter. Some of the globules are as large as 30 micra in diameter. If much water instead of a little is added, the dispersion of the antigen lipid is much greater and the particles are much smaller. If sufficient water is present, the dispersion is so great that the particles in great part are invisible through the microscope. The antigen in water, accordingly, behaves as a hydrophilic colloid.

The preparation of optimal antigen emulsion for tests for syphilis, the lipid is emulsified in a very small quantity of salt solution to insure the presence of the largest possible globules. When this emulsion is added to a strongly positive syphilitic serum, the latter causes a change in the surface of the globules and there results an aggregation of the altered globules. The globules may also be flocculated by alcohol (dehydration effect), by neutral salts, particularly bivalent and trivalent ones, and especially by acids (electrical neutralization or electrical neutralization and dehydration effects). As stated previously, it requires a strongly positive serum to flocculate the antigen globules. With weakly positive serum, flocculation does not occur and accordingly emulsions containing particles of antigen alone are insufficiently sensitive for serodiagnostic tests for syphilis.

In order to make the emulsions sufficiently sensitive, various substances are added to the antigen. In general, cholesterin is employed. Dr. Johns in his excellent slide test uses Balsam of Tolu as does Meinicke. Eagle in his test employs sitosterol as well as cholesterin. These adventitious substances upon

addition of water to their alcoholic solution, precipitate as clumps of crystals and behave characteristically as hydrophobic colloids. Although possessing no antigenic power, they greatly increase the sensitivity of antigen emulsions. As ordinarily employed, cholesterin or like substances is added to alcoholic solution of antigen and this combined solution is mixed with salt solution. As a result particles are formed composed of antigen and cholesterin, apparently uniformly distributed. It has been found that the size and shape of such particles depend especially upon the quantitative relation of the lipoids to water, upon the speed of mixture and upon the temperature of the ingredients. It is possible in chemically identical emulsions, for instance, to produce large needle like particles 60 micra or more in length by slowly mixing cold ingredients, and very small particles, 1 micron and less in diameter, by quickly mixing warm ingredients. Furthermore, because antigen tends to emulsify in water in discrete small globules and cholesterin or like substance tends to precipitate in clumps of crystals, particles produced as just stated are decidedly unstable and actually change in form and composition. In a few minutes to about eight hours at room temperature the changes are so marked that the emulsions are unsatisfactory for use. Within proper time limits, however, these flat particles of antigen and cholesterin or like substance, better adapted for agglutination than antigen globules alone, serve better than the latter in the serodiagnosis of syphilis.

When large needle-like particles are employed, as in the Müller-Ballung reaction, and in the "ball tests" demonstrated in the Scientific Exhibit, their marked alteration by strongly positive serum results in clumps with numerous needle-like projections which interlock upon sufficient agitation forming one large ball in clear fluid. If the particles are less altered, the ball formation is less pronounced and the fluid is somewhat turbid. With negative sera, the tests show a very turbid fluid and no clumps. The advantage of this type of reaction first reported by Hecht, in 1914, and employed by Müller in his Ballung test is that the result may be read accurately and easily with the naked eye. With the "ball tests," it is not necessary to call a conference to decide whether a test is negative or doubtful, doubtful or positive since the different reactions are strikingly distinctive.

Concerning the mechanism of the Kahn test, the Kahn antigen dilution is composed of clumps of numerous small particles averaging about 1 micron in diameter or less. In the presence of positive or negative serum, the fluid present leads to a breaking up of the clumps, the particles becoming discretely distributed in the fluid. If the serum is a positive one, the particles are immediately and intimately surrounded by reagin. The process is somewhat analogous to blotting a section and adding alcohol thereafter instead of adding alcohol to a water-soaked section. (The dehydration is much better than if the alcohol reached the tissue through a film of water.) If the serum is a negative one the particles remain discretely dispersed but if it is a positive one, the

preliminary discrete dispersion of the particles is followed after the surface alteration by a re-aggregation of the altered particles. (In his original test, Kahn employed completely dispersed particles in the antigen dilution. The test, however, required a long time for its completion.) Although the incomplete dispersion of antigen particles determines greater sensitivity by affording optimal conditions for alteration of particle surface by syphilitic serum, it seems more advisable to employ an emulsion of discrete flat particles of larger size requiring less reagin for formation of a visible aggregate. This latter principle is employed in most of the precipitation tests.

Inasmuch as the mixture of cholesterolized antigen solution and salt solution results in emulsions of particles varying greatly in size and shape depending upon the quantity of water, the temperature of ingredients and the speed of mixture particularly, emulsions so prepared are unavoidably variable since there is a definite relationship between the size, shape and number of antigen particles to the sensitivity of the emulsion. In contrast to such emulsions are those prepared by first precipitating the cholesterol in water and subsequently coating the cholesterol plates with antigen lipid. Because the quantity of water, the temperature of the ingredients and the speed of mixture exert no great influence upon cholesterol precipitation in water, the particles so prepared are more uniform in size and shape and when coated with antigen lipid determine emulsions giving more uniformly sensitive results in tests for syphilis. This principle is employed in the preparation of emulsions for the microscopic slide precipitation tests for syphilis. Furthermore, such emulsions are comparatively stable, being satisfactory for use for forty-eight hours.

The rôle of cholesterol in the antigen emulsion is very clearly shown by this method of preparation. Whereas with a fixed quantity of cholesterol in a given total the antigen may be varied as much as 400 per cent without appreciable change in results, the sensitivity of the emulsion is influenced greatly by the amount of cholesterol present. For instance, with 0.1 cc. of antigen in a total of 4.4 cc., a sensitive emulsion is determined by the addition of 0.75 cc. of 1 per cent cholesterol whereas with 1.25 cc. of 1 per cent cholesterol in the same formula a very sensitive emulsion is produced. By varying the quantity of cholesterol, then, the sensitivity of the emulsion may be set at any desired level. If too much cholesterol or other like adventitious substance is present, the emulsion may be too sensitive for reliable diagnostic purposes and the false positivity will be due not to the antigen but to the adventitious material.

Inasmuch as alcoholic tissue extracts contain abundant adventitious material as well as antigen lipid, it has been found necessary for reliable results to remove these substances as much as possible and to add a known quantity of cholesterol or like substance to obtain the desirable sensitivity. Dried tissues accordingly have been extracted with ether or acetone to remove the undesirable substances and the subsequent alcoholic extract has been employed as antigen. Kahn antigen is such a purified antigen. It has been found, however, that whereas

this antigen does not contain appreciable acetone soluble substances, it does contain ether insoluble adventitious substances, the quantity varying with different powders and different ethers employed. A method of more completely purifying antigen is that employed by Noguchi, Browning Cruikshank and McKenzie, Neymann and Gager, Kolmer and Kiss. This method consists in the addition of acetone to alcoholic tissue extract. The adventitious substances are soluble in the acetone and the small acetone insoluble fraction is the specific antigenic portion.

The antigen employed for the microscopic slide precipitation tests is such an acetone insoluble fraction of alcoholic heart powder extract and has been found not only potent and specific but likewise freer of adventitious material than antigen prepared by alcohol extraction of heart powder following preliminary ether or acetone extraction. It serves so well as a base for preparation of antigen emulsions of any desired sensitivity that it may well be worth while for the Society to determine definitely the value of this fraction of alcoholic tissue extract originally recommended by Noguchi.

In the preparation of such an antigen the conditions of the precipitation in acetone are of importance. After most of the alcohol of the tissue extract is evaporated it is poured into acetone. The purest fractions are obtained when the precipitation occurs in acetone at 50°C. to 37°C. for a period of fifteen minutes. Longer periods of precipitation and precipitation at lower temperatures permit of precipitation of adventitious substances as well and such antigens give more sensitive and less specific results.

Concerning the clinical application of serum tests for syphilis, as pointed out by Kolmer and borne out by our experience in early and in treated cases of syphilis, such tests are not sensitive enough rather than too sensitive.

In a study of 9000 tests, for instance, with 20 per cent of the sera from syphilitics, there were eighty-three false negative very sensitive slide tests in treated syphilis and three in primary syphilis and 248 false negative very sensitive Wassermann tests with the same antigen in treated syphilis and seven in primary syphilis. For this reason an even more sensitive slide test is now being employed more surely to exclude syphilis. The additional sensitivity is achieved by heating the antigen emulsion at 56°C., by centrifuging it and by using the larger particles only in proper quantity. With such an emulsion, reactions of some positivity are noted as early as the first week after the appearance of the primary lesion and in treated cases for as long as a year after tests of ordinary sensitivity give negative results.

With modern hospital practice necessitating frequent blood transfusions, the laboratory has the responsibility of preventing the transfer of syphilis by this means and just as it is deplorable that a single false positive blood test should occur to stigmatize an individual so would it be no less deplorable to give syphilis to a single individual by transfusion because of a false negative blood test.

HEMOGLOBIN STANDARDS*

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Estimations of hemoglobin often are unsatisfactory to the laboratory worker and the reports may be confusing to the clinician. Unfortunately, there is no unanimity of opinion concerning the technic of determination or manner of recording results. This confusion is due largely to the multiplicity of methods employed and to the fact that there is now on the market no hemoglobinometer reading in per cent having any scientific basis for the selection of the standard used. The following clinical methods for the estimation of hemoglobin are in use in this country: (1) oxygen combining power (Van Slyke); (2) iron content (Wong); (3) spectrophotometer (Sheard and Sanford); (4) comparison with colored standards: (a) tinted paper (Talquist, (b) glass replacing hemoglobin (Dare, Von Fleischl-Miescher), (c) carbon monoxide-hemoglobin (Haldane, Palmer), (d) acid hematin (original Sahli), (e) solutions to substitute for acid hematin, in the Sahli apparatus or in a Duboscq colorimeter (Felton's pyrogallate and Osgood and Haskins' iron-chromium solutions), and (f) glass to substitute for acid hematin (Bausch and Lomb-Newcomer, Klett, new Sahli, Haden-Hausser).

The oxygen capacity method employing the Van Slyke apparatus is accurate when carried out by an experienced and trained technician but is too complicated to be used as a routine clinical laboratory procedure. The determination of the quantity of iron by the new technic of Wong¹⁸ is accurate, quite simple, and deserves much wider use. The spectrophotometer is expensive, must be calibrated with solutions of unstable crystallized hemoglobin, and can be utilized only as a major laboratory procedure

* Read before the Eleventh Annual Convention of the American Society of Clinical Pathologists, New Orleans, Louisiana, May 6-9, 1932.

even when the human eye is replaced by a photoelectric cell as in the Sheard-Sanford¹² hemoglobinometer. Of the colorimetric methods, the Talquist is little better than guessing; the Dare is difficult to read accurately, and subject to wide errors; the Sahli with an acid hematin, yellow glass or correctly made artificial standard (Osgood-Haskins) is a good clinical method; the Duboseq colorimeter with a colored glass standard as in the Newcomer and Klett methods or with correct standard solutions is satisfactory for laboratory use; and the Haden-Hausser instrument has its place of usefulness.

TABLE 1
HEMOGLOBIN STANDARDS IN DIFFERENT HEMOGLOBINOMETERS READING IN PER CENT

| INSTRUMENT | GRAMS EQUAL TO 100 PER CENT HEMOGLOBIN |
|--|--|
| Haldane..... | 13.80 |
| Oliver..... | 15.00 |
| Talquist..... | 15.80 |
| Dare (prior to 1926)..... | 13.80 |
| Dare (1926 to 1931)..... | 16.90 |
| Dare (1931 to —)..... | 16.00 |
| Bausch and Lomb-Newcomer (original)..... | 16.90 |
| Sahli (original)..... | 17.20 |
| Sahli (present)..... | 14.00 |
| Von Fleischl-Miescher..... | 15.80 |
| Klett..... | 15.36 |

The estimation of oxygen capacity by means of the Van Slyke apparatus is the method of choice for research work and for determining and checking standards. The iron method is the only possible substitute; comparative estimations indicate that it gives results equally as accurate as the oxygen capacity method (table 1). Where an inexpensive laboratory instrument is required, the Sahli with a solid glass rod standard, or with the Osgood-Haskins¹⁰ artificial standard is satisfactory, if calibrated in grams. The Haden-Hausser⁴ hemoglobinometer is simple and accurate but unfortunately more expensive. For use only in the laboratory the Bausch and Lomb-Newcomer and Klett instru-

ments with glass or liquid standards are very good. The accuracy of all the colorimetric methods cited, however, is almost entirely dependent upon the calibration of the glass or solution used as a standard, and can be depended upon only when this has been done correctly.

With the oxygen capacity and the iron methods the hemoglobin readings are directly in grams. Until recently most hemoglobinometers have yielded readings in per cent with no two instruments in agreement as to what should be taken as equivalent to 100 per cent (table 2). In later models of the Von Fleischl-Miescher

TABLE 2
HEMOGLOBIN IN GRAMS PER 100 CC.

| NUMBER | OXYGEN CAPACITY METHOD (VAN SLYKE) | IRON CONTENT METHOD (WONG) |
|--------------|---------------------------------------|----------------------------|
| | <i>grams</i> | <i>grams</i> |
| 1 | 13.13 | 13.46 |
| 2 | 15.11 | 14.68 |
| 3 | 15.02 | 14.68 |
| 4 | 13.28 | 12.95 |
| 5 | 17.31 | 17.53 |
| 6 | 15.82 | 16.25 |
| 7 | 14.95 | 14.63 |
| 8 | 13.15 | 12.98 |
| 9 | 12.40 | 12.50 |
| 10 | 13.43 | 13.32 |
| Average..... | 14.36 | 14.30 |

and the new Bausch and Lomb-Newcomer apparatus, the amount of hemoglobin is determined in grams only, although figures given in the table are suggested for transposing the figures into per cent. The Klett and the new Sahli with solid glass rod standard give readings in grams as well as in per cent; the results with the Haden-Hausser instrument are calculated only in grams. The Von Fleischl-Miescher instrument is calibrated by comparison with a solution of unstable hemoglobin crystals; the new Bausch and Lomb-Newcomer by spectrophotometric determinations, and the Haden-Hausser by direct Van Slyke readings; the method of calibration of the Klett and Sahli instruments is not known to me.

It is apparent that with proper calibration any accurate colorimetric method can be transposed directly into grams. Thus Osgood and Haskins'^{7,9} results in normal individuals reported in grams were obtained by calibrating their colorimetric procedure by direct comparison with readings on the Van Slyke instrument. Wintrobe's figures^{16,17} were obtained with a Newcomer glass standard checked by the oxygen capacity method. It seems fair to require that all hemoglobinometers, reading either in per cent or in grams should have as their basis of calibration figures

TABLE 3

COMPARISON OF HEMOGLOBIN READINGS WITH OXYGEN CAPACITY AND IRON CONTENT METHODS AND HADEN-HAUSSER HEMOGLOBINOMETER

| BLOOD NUMBER | OXYGEN CAPACITY METHOD (VAN SLYKE) | IRON METHOD (WONG) | HADEN-HAUSSER HEMOGLOBINOMETER |
|------------------|---------------------------------------|--------------------|-----------------------------------|
| | <i>grams*</i> | <i>grams*</i> | <i>grams*</i> |
| 1 | 12.1 | 11.8 | 12.0 |
| 2 | 12.4 | 12.1 | 12.0 |
| 3 | 10.6 | 10.3 | 10.5 |
| 4 | 10.4 | 10.7 | 10.5 |
| 5 | 9.4 | 9.4 | 9.5 |
| 6 | 7.6 | 7.8 | 7.8 |
| 7 | 14.4 | 14.4 | 14.5 |
| 8 | 6.0 | 6.2 | 6.0 |
| 9 | 8.0 | 8.4 | 8.0 |
| 10 | 11.2 | 11.8 | 11.5 |
| Average. | 10.23 | 10.29 | 10.23 |

* Grams per 100 cc.

obtained by direct Van Slyke readings or a determination of the iron content. Any hemoglobin determination would thus be designated or understood in the laboratory: hemoglobin = — per cent (— gms. by the direct Van Slyke or the iron content method); or hemoglobin = — gms. (direct Van Slyke or iron content method) with the — hemoglobinometer.

In table 3 is shown a series of determinations with the Haden-Hausser hemoglobinometer compared with both the oxygen capacity and iron methods. Such a comparison should be available for every hemoglobinometer used. So far as the technic of

hemoglobin estimations is concerned, one may say that several methods and instruments are satisfactory, but a method or instrument should only be employed which allows the translation of the readings into grams of hemoglobin as determined by the direct Van Slyke or the iron method. This point could easily be settled by common consent among laboratory workers.

The next problem concerns the amount of hemoglobin per hundred cubic centimeters of blood in normal individuals when determined by an accurate method. Haldane,⁵ on the basis of a very small number of determinations found an average of 13.8 grams of hemoglobin in healthy normal men in England. He employed the oxygen capacity method in the Haldane blood-gas apparatus. This so-called Haldane standard (13.8 grams = 100 per cent) is frequently used in America, although every one has realized that normal men show a reading well over 100 per cent when this standard is used. Recently, Price-Jones¹² has found an average hemoglobin content of 112 per cent in normal men in Boston with this standard, using a colorimetric procedure calibrated by the gasometric method. The Haldane standard should never be used in this country, since with its use normal individuals will not have a color index of 1.00. The Williamson¹⁴ standard should also be discarded since the average normal for men (16.92 grams) is much higher than other workers have found with other accurate methods available. The spectrophotometer used by Williamson was calibrated with hemoglobin crystals which are unstable and difficult to prepare in pure form. Hence the results obtained with it can not be compared with the most accurate clinical method (direct Van Slyke) now in common use. Williamson's table is of great value, however, in showing the relative hemoglobin content at different ages in the two sexes.

What is the hemoglobin content per hundred cubic centimeters of normal individuals in this country by the direct Van Slyke or iron content method? No large series of normals has been reported with the iron method. Using the oxygen capacity method I³ found the average in forty normal men several years ago to be 15.4 grams. This series I have recently increased to seventy and for this number the average hemoglobin content is

15.34 grams per hundred cubic centimeters (table 4). Using the same method, Dill² found the average in forty men in Boston, 15.38 grams per hundred cubic centimeters and Sopkey¹³ in India, 15.30 grams in a series of 121 men. These three series are the only ones reported in which all the determinations were done by the oxygen capacity method. Osgood and Haskins (Portland, Oregon) using their inorganic standard calibrated against direct Van Slyke readings reported an average hemoglobin value of 16 grams in 153 men.⁶ Wintrobe and Muller (New Orleans) first reported an average of 15.85 in men using the Newcomer method,

TABLE 4
A SUMMARY OF THREE SERIES OF SIMULTANEOUS ESTIMATIONS OF HEMOGLOBIN
AND RED CELLS IN BLOOD

| OBSERVER AND DATE | DETERMINATIONS | | MEAN RED CELL COUNTS | | HEMOGLOBIN | | | | | |
|---------------------------------------|----------------|-------|----------------------|-------|--------------------|-------|-------------|--------|---------------|--|
| | Men | Women | Men | Women | Mean concentration | | Coefficient | | | |
| | | | | | Men | Women | Men | Women | Men and women | |
| | | | | | | | grams† | grams† | grams† | |
| Osgood and Haskins* (1926-27)..... | 153 | 100 | 5.60 | 4.80 | 16.0 | 13.69 | 14.29 | 14.29 | 14.29 | |
| Wintrobe* (1929-30)..... | 100 | 50 | 5.85 | 4.93 | 17.0 | 13.76 | 14.53 | 13.97 | 14.25 | |
| Haden (1922 and 1932)... | 70 | 30 | 4.95 | 4.38 | 15.34 | 13.37 | 15.50 | 15.26 | 15.35 | |

* Figures given in personal communication from authors.

† Grams per 100 cc.

calibrated by direct Van Slyke readings. Later Wintrobe^{1,5} revised his figures to 17 grams in men since a mistake in calibration of his instrument had been found. The figures for women are, of course, lower. Figures reported for other parts of the world vary little from those cited.

The clinician is interested in the absolute values for hemoglobin in the blood, but is even more interested in the hemoglobin content relative to normal and in the amount relative to the number of red cells present. Such reports are of great value and are accurate, provided one can translate them at will into grams, which is always possible if an absolute figure is taken as 100 per

cent. The mean concentration of hemoglobin in normal men should be taken as 100 per cent. It is apparent that a different figure would have to be taken for women. In studying the various types of anemia, the clinician must know the relation of the amount of hemoglobin to the red cell count, namely, the color index, and in calculating the per cent of cells, 5,000,000 red cells are taken as 100 per cent of cells. It is thus necessary to take as 100 per cent for the hemoglobin the number of grams of hemoglobin found per hundred cubic centimeters of blood per five million cells (the hemoglobin coefficient). In determining a standard the erythrocyte count as well as the amount of hemoglobin must be known. Haldane and Williamson did not record the number of red blood cells. The Haldane and Williamson standards have already been discussed as impossible of acceptance on other grounds but this is an added reason why they should not be used. Unfortunately, also, no red cell counts are reported for the hemoglobin estimations of Dill. Only three large series of simultaneous estimations of hemoglobin and of red cells have been made in this country, those of Osgood and Haskins, (1926-1927), of Wintrobe (1929-1930) and of Haden (1922 and 1932). A summary of the findings in these three series is shown in table 4. Osgood and Haskins found the same hemoglobin coefficient in men and women (14.29 grams); Wintrobe found 14.53 grams in men and 13.97 in women; my results show 15.50 grams in men and 15.26 grams in women. The difference in hemoglobin per cell in men and women is small so it is unnecessary to use a different standard for the two sexes.

It is apparent from table 4 that the variation in the hemoglobin coefficient is due largely to differences in the erythrocyte counts. If all the figures are correct, apart from the variation in hemoglobin, there is evident a wide variation in red cell count in different parts of the country. This might well be, since with the higher red cell counts reported for the Scandinavian countries, the hemoglobin is much the same as in this country. I have found the number of red cells in normal men in Detroit (5,030,000), in Kansas City (4,880,000), and in Cleveland (5,030,000) approximately the same. Likewise I found that red cell counts done by medical

students in Kansas City on their own blood with Hausser-Levy counting chambers varied little from year to year as is indicated in table 5. Sixteen female students during this same period showed an average of 4,420,000, per cubic centimeters. Emerson¹ reported an average of 5,000,000 among 176 medical students in Baltimore. No large series have been reported from other centers.

The hemoglobin standard which I suggested in 1922 as 100 per cent was 15.6 grams (15.55 grams). I am now using 15.4 grams on the basis of the larger series (100 men and women) reported here, although there is actually a difference of only 0.12 gram per hundred cubic centimeters in the standard for the two groups.

TABLE 5
RED CELL COUNTS MADE BY MEDICAL STUDENTS IN KANSAS CITY

| YEAR | NUMBER OF STUDENTS | AVERAGE COUNT |
|------------|--------------------|----------------|
| 1924-1925 | 25 | 5.12 |
| 1925-1926 | 37 | 5.06 |
| 1926-1927 | 32 | 5.04 |
| 1927-1928 | 36 | 4.99 |
| 1928-1929 | 46 | 5.02 |
| 1929-1930 | 54 | 4.85 |
| Total: 230 | | Average: 4.997 |

My standard has been found to give normal color indices for other workers in Philadelphia, Boston, and Baltimore, and for me in Detroit, Kansas City and Cleveland.

How is one to explain the differences in the three reported series, each suggesting a different hemoglobin coefficient? This can not be done. Only further accurate studies in different parts of this country will determine to what extent the normal red cell count varies. Until some common figure is agreed upon, the selection of a single standard is impossible. Tables such as Osgood⁸ has given for calculating percentages can not be universally used since they are correct only when the red cell counts and the hemoglobin are the same as he has found them in Portland.

It is necessary that some method be made available for reporting to clinicians the hemoglobin in per cent and for calculating accurately the color and saturation indices. To meet this need I have the following plan to offer. Only a method or instrument for the determination of hemoglobin should be used which reads in grams as calibrated by the direct Van Slyke or iron content method and all readings should be recorded in the laboratory in grams. In each laboratory, then, the mean value for hemoglobin and for the red cell count is determined for the place in which the laboratory is located. This is done easily and quite accurately by counting the red cells in ten or more normal adults and by determining simultaneously the hemoglobin in the same individuals with an instrument reading in grams per hundred cubic centimeters. The mean value for erythrocytes and for hemoglobin is thus found and the mean value for hemoglobin in grams per hundred cubic centimeters of blood per five million cells or per million cells is calculated.

The number of grams of hemoglobin so determined is taken as normal or 100 per cent for the laboratory in which the calibration is done. When so determined the hemoglobin percentage in a given blood will be the same everywhere and with every observer even though there may be a wide variation in absolute figures, and the color index in normal individuals always will be within normal limits (0.90 to 1.10). Using this plan, a hemoglobin report would read: hemoglobin = — per cent (100 per cent = hemoglobin content of normal adult with 5,000,000 erythrocytes). If, on further investigation, it is found that the hemoglobin coefficient is actually everywhere the same, absolute figures can then be used. Until such a time, each worker may use a standard hemoglobin coefficient determined on the basis of hemoglobin estimations and red cell counts made in his own laboratory, and may report the amount of hemoglobin in per cent of this standard.

SUMMARY AND CONCLUSIONS

1. In making hemoglobin determinations only an instrument or method should be employed in which the results are recorded in

grams as determined by the oxygen capacity or iron content method.

2. For clinical purposes the reports of hemoglobin are best given in per cent of normal of a healthy adult with a red cell count of 5,000,000 cells.

3. The Haldane and Williamson standards can not be so transposed since in determining the standards no red cell counts were done.

4. There is a wide variation in hemoglobin and erythrocyte counts and consequently in the hemoglobin coefficient in the three large series (Haden, Osgood and Haskins, and Wintrobe) reported for normal men and women.

5. A satisfactory standard must give a color index within normal limits (0.90 to 1.10) in normal individuals.

6. In the present state of confusion, the hemoglobin coefficient should be determined for each laboratory and the hemoglobin should be reported only in per cent of this normal.

7. The hemoglobin percentage of a given blood when determined by this method is necessarily the same in all laboratories within the limits of technical error.

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EDITORIAL

THE FRIEDMAN HORMONE TEST FOR PREGNANCY

Although evidence of and tests for pregnancy have been studied for centuries, credit should be given to Aschheim and Zondek, who in 1928 proposed an accurate experimental method of determining this condition. The method required that five or six immature, female, white mice, twenty-one days old, be given injections of varying quantities of urine of the woman in question. Injections were given subcutaneously on two successive days, three times a day. After 100 hours, the ovaries were examined for precocious corpora lutea and corpora hemorrhagica, their presence being an indication that the urine contained an excess of anterior pituitary hormone and therefore that the woman was pregnant.

The accuracy of this method has been confirmed repeatedly, and the test now rests among the most refined known in biology. Unfortunately the method is relatively tedious and requires access to a larger colony of mice than is generally available.

Friedman, a little more than a year after the important work of Aschheim and Zondek had been done, profiting by his knowledge of the peculiar nature of ovulation in the rabbit, devised a much simpler test for pregnancy. He injected 5 cc. of urine from pregnant woman into young, adult, female rabbits, and was able to demonstrate that this urine produced typical corpora hemorrhagica in the ovaries of the rabbits. Urine from nonpregnant women produced no such results.

Results of the Friedman test were first presented before a scientific assembly at the ninth annual convention of the American Society of Clinical Pathologists, by Reinhart and Scott. Their results were excellent. This presentation was followed by numerous reports which amply confirmed their observations. The society has therefore felt a peculiar interest in this test, and

TABLE 1
FRIEDMAN HORMONE PREGNANCY TESTS

| TOTAL TESTS | POSITIVE | NEGATIVE | FALSE POSITIVE | FALSE NEGATIVE | ECTOPIC—POSITIVE | ECTOPIC—NEGATIVE | CHORIOEPITHELIOMA OR HYDATID MOLE— POSITIVE | CHORIOEPITHELIOMA OR HYDATID MOLE— NEGATIVE | EARLIEST TEST, DAYS AFTER LAST MEN- STRUATION | AMOUNT OF URINE (EACH INJECTION) | NUMBER OF INEC- TIONS | DURATION OF TEST hours | ANIMALS DYING FROM INJECTIONS | CLINICAL PATHOLOGIST REPORTING |
|------------------|----------|----------|----------------|----------------|------------------|------------------|---|---|---|-------------------------------------|--------------------------|------------------------------|----------------------------------|---|
| | | | | | | | | | | | | | | |
| 27 | 18 | 9 | | 2 | 1 | 1 | | | 35 | 10 | 1 | 36 | 1 | M. D. Bell, Dallas, Texas |
| 32 | 10 | 16 | 1 ¹ | 1 | | 1 | | | 56 | 10 | 1 | 48 | 2 | E. D. Funk & F. M. Light, Reading Hospital, Reading, Pa. |
| 21 | 13 | 6 | 2 | | 2 | | | | 32 | 7-10 | 1 | 30 | | H. F. Hunt, Geisinger Memorial Hospital, Danville, Pa. |
| 96 | 56 | 40 | | | 1 | | | | 35 | 12 | 1 | 24 ² and 48 | 2 | L. C. Todd, Charlotte, N. C. |
| 16 | 7 | 9 | 1 | 1 | | | | | 22 | 7-20 | 1 | 48 | 2 ¹ | B. C. Shakford, Long Beach, Cali- fornia |
| 40 | 18 | 22 | 1 ³ | | | | | | 10 | 10 | 1 | 48 | | J. M. Moore, San Antonio, Texas |
| 102 ⁴ | 55 | 47 | 3 | 2 | | 1 | | | 10 | 7-12 | 1 | 30 | 2 | B. C. Russum, Omaha, Nebraska |
| 46 | 26 | 20 | 17 | 1 | | | | | 4 | 10 | 1 | 48 | 4 | J. C. Simpson, Morristown, Pa. |
| 57 | 28 | 29 | 1 | 1 | | | | | 33 | 5-10 | 1 | 36 | 3 | J. R. Van Atta, Albuquerque, New Mexico |
| 10 | 10 | 10 | | | | | | | | 7-10 | 1 | 30-40 | | T. Zbinden, Private Laboratory |
| 34 | 30 | 3 | 1 ⁴ | | | | | | 42 | 7 | 1 | 48 | | G. B. Kramer, Youngstown, Pa. |
| 104 | 37 | 67 | 4 ² | | 1 | | | | 14 | 7-10 | 1 | 30 | 3 | J. E. Robinson, King's Daughters Hospital |
| 74 | 31 | 43 | | | | | 1 | | 12 | 12-20 | 1 | 48 | 1 | M. B. Lyon, South Bond, Indiana |

| | | | | | | | | | | | | | |
|-----|-----|-----|---|---|---|---|---|-------------------------|-------|---|-------|---|---|
| 181 | 87 | 94 | 3 | 4 | 2 | 1 | 1 | 29 | 8-12 | 1 | 48 | 1 | F. C. Payne, Dayton, Ohio |
| 363 | 188 | 175 | | 4 | 3 | 1 | 1 | 20 (after coitus) | 10 | 2 | 39 | 6 | G. D. Maner, Los Angeles, California |
| 44 | 26 | 18 | | | 1 | | 1 | 5 | 10 | 2 | 48 | 3 | A. S. Giordano, South Bend, Indiana |
| 95 | 56 | 39 | | 1 | 1 | | | 21 (after coitus) | 10 | 2 | 48 | 1 | B. W. Rhumy, Fort Wayne Med. Laboratory |
| 112 | 78 | 34 | | 1 | 1 | 1 | | 17 | 10 | 2 | 36-48 | 4 | V. Cofain, Seattle, Washington |
| 14 | 5 | 9 | | | | | | 42 | 16 | 1 | 30-36 | 2 | C. R. Drake, Minneapolis, Minnesota |
| 75 | 49 | 26 | | | 1 | | 1 | | 6 | 2 | 48 | | L. P. Hastings, Hartford, Connecticut |
| 65 | 36 | 29 | | | 1 | | | 33 | 10-20 | 1 | 24-48 | 3 | May Owen, Terrell Laboratories |
| 135 | 54 | 78 | 2 | 1 | 2 | 4 | 1 | 8 | 10 | 2 | 48 | 2 | W. Simpson, Dayton, Ohio |
| 369 | 134 | 235 | 1 | 9 | | | | 33 | 24 | 1 | 48 | | Gertrude Moore, Oakland, California |
| 60 | 31 | 28 | | 1 | | 1 | 1 | 1 ¹⁰ | 8-10 | 4 | 36-48 | 3 | A. G. Foord, Pasadena, California |
| 109 | 45 | 60 | 1 | 1 | 1 | 1 | | 30 | 10-20 | 1 | 48 | 4 | B. S. Kline, Mt. Sinai Hospital, Cleveland, Ohio |
| 24 | 15 | 9 | | | | | 1 | 28 | 10 | 2 | 30-48 | | E. M. Butt, Clinical Laboratory, Los Angeles, California |

¹ Result uncertain at the time.

² Surgeon reported ovum had been dead for at least one month.

³ Two rabbits for each test.

⁴ One specimen from patient taking ergotin compound.

⁵ Second specimen was positive.

⁶ 59 were Aschheim-Zondek tests.

⁷ Probably not false as abortion probably took place.

⁸ Patient had dead fetus.

⁹ Probably due to being unable to control collection of certain specimens.

¹⁰ After operation, patient well after six months.

TABLE 1—Concluded

| TOTAL TESTS | POSITIVE | NEGATIVE | FALSE POSITIVE | FALSE NEGATIVE | ECTOPIC—POSITIVE | ECTOPIC—NEGATIVE | CHORIOEPITHELIOMA OR HYDATID MOLE— POSITIVE | CHORIOEPITHELIOMA OR HYDATID MOLE— NEGATIVE | EARLIEST TEST, DAYS AFTER LAST MEN- STRUATION | AMOUNT OF URINE (EACH INJECTION) | NUMBER OF INJECTIONS | DURATION OF TEST <i>hours</i> | ANIMALS DYING FROM INJECTIONS | CLINICAL PATHOLOGIST REPORTING | |
|---------------------|----------|----------|-----------------|----------------|------------------|------------------|---|---|---|-------------------------------------|----------------------|----------------------------------|----------------------------------|--------------------------------|---|
| 24 | 12 | 12 | | | | | | | | cc. | 7 | 1 | 48 | | W. M. Sheppo, Wheeling Clinic, Wheeling, West Virginia |
| 141 ¹¹ | 66 | 75 | 2 | 4 | | | 1 | | 7 | 4 | 6 ¹² | 48 | 3 | | J. H. Littener, Nashville, Tennessee |
| 8 ¹³ | 4 | 4 | | | | | | | | 7 | | 35 | | | W. H. Bailey, Wesley Hospital, Oklahoma City, Oklahoma |
| 204 | 92 | 112 | 3 ¹⁴ | 3 | 3 | | | | 20 (after coitus) | 5-20 | 1 | 24-48 | 6 | | T. B. Magath, Mayo Clinic, Rochester, Minnesota |
| 865 | 423 | 442 | 5 | 15 | | | 3 | | | 10 | 1 | 48 | | | H. L. Reinhart, Ohio State University, Columbus, Ohio |
| 1,048 ¹⁵ | 790 | 258 | | 48 | 68 | 12 | 6 | | 14 (after coitus) | 10 | 1 | 48 | 10 | | F. E. Sondern & I. J. Silverman, New York City |
| 4,595 | 2,526 | 2,058 | 24 | 106 | 88 | 25 | 18 | 1 | | | | | 72 | | |

¹¹ 61 were Aschheim-Zondek tests.¹² Three times a day.¹³ Aschheim-Zondek tests also used.¹⁴ One false positive in patient with suprarenal tumor.¹⁵ Aschheim-Zondek tests on 487 simultaneously with Friedman as well as on many others in this series.

at the eleventh convention, authorized Dr. A. G. Foord, Chairman of the Committee on Research, to send out a questionnaire to all members of the Society requesting information as to their experience with this test. This information may be found in table 1, from which certain definite conclusions can be drawn. More extended consideration of the test will be found in two articles in this issue.

Although there have been many slight modifications of the procedure advocated by Friedman, it becomes evident that the test is satisfactorily performed when about 10 cc. of freshly passed, morning urine is injected into the marginal ear vein of a young, nonpregnant adult, female rabbit which has been isolated from male rabbits for at least three weeks. The observation of the ovaries of the rabbit is best made after forty-eight hours has elapsed, although after twenty-four hours the reaction is usually sufficient to make an observation trustworthy. To allow observation of the ovaries, the animal may be killed, or it may be operated on under anesthesia. If operation is performed the rabbit may be used again after one month.

Since information has been obtained on 4,595 tests, of which 55 per cent were positive and 45 per cent negative, one may reach very definite conclusions as to the value of the test. It is demonstrated, for instance, that 130 (2.83 per cent) results were erroneous. Unfortunately, as in the case of most questionnaires, the information given was not entirely accurate, since all observers probably did not report on exactly the same basis. There is, therefore, a very small percentage of error in these figures. Nevertheless, the largeness of the sample makes the reported results highly accurate. The tests which gave erroneous results are divided so that twenty-four (0.95 per cent) are false positives and 5.1 per cent are false negatives. Many perfectly logical explanations are offered for these errors. The most common false negative result is due to testing the urine before the woman has been pregnant long enough for the result to be positive in the rabbit. Other sources of error are failure to inject fresh urine, and failure to use sufficient quantities of urine. Some of the false positive results are due to failure to have the test rabbit

isolated from male rabbits, and perhaps occasionally to erroneous interpretation of the reaction. But any procedure which in a field test yields an uncorrected accuracy of more than 97 per cent, must be considered extremely satisfactory.

It was hoped that this test would yield accurate results in regard to ectopic pregnancy, in which condition accurate information is extremely important. Realizing the difficulties of verifying all such tests from a clinical and surgical standpoint, it is gratifying to note that the test has yielded an accuracy of 77.8 per cent, which, although the degree of accuracy is not so great as in uterine pregnancy, nevertheless compares very favorably with clinical diagnoses.

In the presence of chorio-epithelioma and hydatid mole the test gives extremely satisfactory results. There were nineteen such cases, in eighteen of which the test was positive. In regard to unusual malignant conditions of the testes, supposed to take origin from ovarian rests, information is not available.

An important consideration in hormone tests for pregnancy is the earliness with which the test will give a positive result. From the information available it becomes evident that the urine might contain sufficient hormone to produce a positive reaction in as short a time as fourteen days after impregnation. It seems quite evident that the test will be positive after three weeks.

From available information one must assume that about 1.5 per cent of the animals used will die, resulting in the failure of the test.

The Committee on Research and the Editor take the greatest pleasure and gratification in reporting these results, and thank the members of the Society who coöperated in making the report possible. It is evident that as a single laboratory procedure, on the basis of this rather large number of tests made by many different observers, the Friedman hormone test for pregnancy must be considered an outstanding contribution to the practice of medicine.

NEWS AND NOTICES

TRAINING SCHOOLS FOR TECHNICIANS

The information contained in the tables on pages 104-106 deals with hospital and post-graduate training schools for technicians which have been approved through registration by the committee on Registry. It is furnished by Dr. Kano Ikeda and will be of great interest to clinical pathologists and laboratory technicians. Other applications are pending now and will be acted upon at the next annual convention.

CORRECTION OF ERRORS

The Editor wishes to call attention to two unfortunate errors in the list of members as published in the November issue of the JOURNAL. Dr. F. E. Sondern should have been indicated as a Counsellor and Dr. A. V. St. George, who is a Fellow of the Society, should not have been indicated as an Associate Member.

COMMITTEE APPOINTMENTS

The following Committees have been appointed:

Committee on Scientific Exhibit Awards

Dr. W. S. Thomas, Chairman
Dr. E. L. Miloslavich
Dr. H. C. Sweany

Program Committee

Dr. A. S. Giordano, Chairman
Dr. A. H. Sanford
Dr. F. W. Hartman

Committee on Local Arrangements

Dr. N. Enzer, Chairman (Hotel Accommodations)
Dr. E. L. Tharinger (Publicity)
Dr. E. F. Barta (Commercial Exhibits)
Dr. M. Fernan-Nunez (Scientific Exhibits)

Publication Committee

Dr. J. A. Kolmer, Chairman
Dr. Kano Ikeda
Dr. W. C. MacCarty

Research Committee

Dr. A. G. Foord, Chairman
Dr. B. S. Kline (Serology)
Dr. R. R. Kracke (Hematology)
Dr. O. A. Brines (Pathologic Anatomy)
Dr. I. A. Nelson (Biochemistry)
Dr. Ruth Gilbert (Bacteriology)
Dr. B. F. Stout (Vaccines)

Necrology Committee

Dr. J. J. Moore, Chairman
Dr. Herman Spitz
Dr. F. C. Payne

Committee on Necropsies

Dr. F. E. Sondern, Chairman
Dr. P. Hillkowitz
Dr. I. Davidsohn

Round Table Committee

Dr. A. G. Foord, Chairman
Dr. L. W. Larson
Dr. W. M. Sheppe

DATA ON TRAINING SCHOOLS FOR TECHNICIANS APPROVED THROUGH REGISTRATION BY THE BOARD OF REGISTRY

| SCHOOL | ADDRESS | DIRECTOR | ENTRANCE REQUIREMENTS | LENGTH OF COURSE | FEE'S |
|--|---------------------------------|-----------------------|--|------------------|----------|
| Aneker Hospital Laboratory... | St. Paul, Minnesota | Dr. John F. Noble | 2 years university work | 1 year | None |
| Archbold Memorial Hospital Laboratory..... | Thomasville, Georgia | Dr. M. J. Erickson | 1 year college work | 1 year | None |
| *Atlantic City Hospital Laboratory..... | Atlantic City, N. J. | Dr. R. A. Kilduffe | High school diploma | 12-16 mo. | None |
| Bender Hygienic Laboratory... | Albany, New York | Dr. A. W. Wright | 1-2 years college work | 1 year | \$100.00 |
| Buffalo General Hospital Laboratory..... | Buffalo, New York | Dr. Byron D. Bowen | College work | 1 year | None |
| Henry Ford Hospital..... | Detroit, Michigan | Dr. F. W. Hartman | Degree from app. college or university | 18 mo. | None |
| Dr. W. G. Gamble's Training School..... | Bay City, Michigan | Dr. W. G. Gamble | 2 years college | 12-18 mo. | \$200.00 |
| Training School Drs. Hillkowitz and Freshman..... | Metrop. Bldg., Denver, Colorado | Dr. Philip Hillkowitz | 1 year college work | 1 year | \$100.00 |
| *Dept. of Laboratories, House of Good Samaritan..... | Watertown, N. Y. | Dr. H. N. Cooper | High school graduate | 18 mo. | None |
| Clinical Laboratory of Jefferson Hospital..... | Philadelphia, Pa. | Dr. B. L. Crawford | 1 year college work | 12-18 mo. | None |
| Minneapolis General Hospital. | Minneapolis, Minnesota | Dr. N. H. Lufkin | 2 years college work | 1 year | \$50.00 |
| Charles T. Miller Hospital..... | St. Paul, Minnesota | Dr. Kano Ikeda | 1 year college work | 16 mo. | \$125.00 |
| *Monmouth Memorial Hospital. | Long Branch, N. J. | Dr. C. A. Pons | High school diploma | 1 year | None |
| *Mount Sinai Hospital..... | Cleveland, Ohio | Dr. B. S. Kline | High school diploma | 1 year | None |
| *Mount Sinai Hospital..... | Philadelphia, Pa. | Dr. D. R. Moranzo | High school diploma | 18 mo. | \$125.00 |
| Newark Beth Israel Hospital... | Newark, N. J. | Dr. Aslier Yaguda | 1 year college | 1 year | None |
| Saint Joseph Hospital..... | Lexington, Ky. | Dr. E. S. Maxwell | 1 year college | 1 year | \$50.00 |
| Stuart Circle Hospital Labs... | Richmond, Va. | Dr. Regenn C. Book | 2 years college | 1 year | \$10.00 |

| | | | | | |
|--|---|----------------------|---------------------------------|---------|----------------------------|
| Swedish Hospital School of Technicians..... | 914 So. 8th St., Minneapolis, Minnesota | Dr. Chas. R. Drake | 1 year college | 1 year | \$100.00 |
| Uniontown Hospital..... | Uniontown, Pa. | Dr. H. A. Heise | 1 year college | 2 years | Students paid \$25 per mo. |
| *Univ. of California Hospital.. | San Francisco, Calif. | Dr. I. C. Schumacher | High school diploma | 16 mo. | None |
| *Graduate Hospital Univ. of Pennsylvania | Philadelphia, Pa. | Dr. John A. Kohner | High school or college graduate | 18 mo. | \$180.00 |
| Wm. Pepper Laboratory of Clinical Medicine | Philadelphia, Pa. | Dr. Herbert Fox | 1 year college | 1 year | \$150.00 |
| *Wm. Volker Laboratory Training School | Kansas City, Mo. | Dr. Fred. C. Narr | High school diploma | 1 year | None |
| Wisconsin General Hospital... | Madison, Wisconsin | Dr. W. D. Stovall | College graduate | 1 year | \$25.00 |
| Presbyterian Hospital | Denver, Colorado | Dr. Paul C. Carson | 2 years college | 1 year | None |
| St. Mary's Hospital | Duluth, Minnesota | Dr. Geo. Berdez | 2 years college | 1 year | \$50.00 |
| St. John's Hospital | Springfield, Illinois | Dr. Walter G. Bain | **Graduate nurse | 1 year | \$50.00 |
| Sacred Heart Hospital | Spokane, Washington | Dr. M. M. Patton | 1 year college | 1 year | None |

* Entrance requirements to be advanced to 1 year college work in chemistry and biology.

** Graduation in nursing to be considered the equivalent of 1 year college in chemistry and biology.

POST-GRADUATE OR COLLEGE COURSE IN MEDICAL TECHNOLOGY REGISTERED WITH THE BOARD

| SCHOOL | ADDRESS | DIRECTOR | ENTRANCE REQUIREMENTS | LENGTH OF COURSE | FEES |
|--|--------------------------------|---------------------|----------------------------------|---------------------------------------|----------------------------|
| Graduate School, Emory University..... | Emory University, Ga. | Dr. Roy R. Kracke | A.B. or B.S. | 12-15 mo. | \$40.00 per major |
| Michigan State College of Agriculture & Appl. Science. | East Lansing, Michigan | Dr. Ward Giltner | High school diploma | 4 years B.Sc. | Reg. College Fees \$250.00 |
| N. Y. Post Grad. Med. School... | 303 E. 20th St., New York City | Dr. Ward MacNeal | College graduate, graduate nurse | 6 mo. (Cert. to M.D.'s only) 90 hours | |
| N. Y. Univ. Med. College..... | 338 E. 26th St., New York City | Dr. Samuel A. Brown | B.A. or B.S. | | |
| North Carolina College for Women..... | Greensboro, N. C. | Lila Belle Love | College entrance | 4 years | Reg. College Tuition |

BOOK REVIEWS

Laboratoriumstechnik in der Medizin. BY NUMEROUS AUTHORS, EDITED BY KURT HOLM. Pp. xii + 714, 1932, Hamburg, Paul Hartung, 36m.

This volume has been prepared by twenty-one different authors whose contributions have been edited and arranged by Holm. It presents the laboratory side of medicine in a manner which is rather novel. The twenty chapters deal with all the various phases of clinical laboratory work, but there are special chapters on physical, chemical, histological and anatomical considerations not usually found in such manuals.

The topics covered by the chapter headings cannot be considered with any degree of detail within the bulk of the volume. However, enough detail is given so that the laboratory technician, for whom the volume has been primarily designed, is enabled to obtain quite a comprehensive general knowledge of the various questions concerned. The technical methods described are presented in a clear concise fashion. In a number of particulars the technic of individual examinations vary from the commonly accepted routine as practiced in the United States.

The illustrations, which are numerous, are for the most part original. They are well done and clearly illustrate the points in question.

The volume is of value to the laboratory technician who, while being familiar with the mechanical routine of laboratory procedures, desires to know more of the theory upon which the procedures are predicated. For those whose education has been more extensive in the chosen fields, the volume is of value in presenting under one cover a mass of information usually found only after search in many scattered volumes. The book, however, does not present the detailed description of methods and technic which would render it of value to advanced workers as an

additional reference book, but this was not the intention of the authors.

—G. L. ROHDENBURG

A Text-book of Pathology: An Introduction to Medicine. BY WILLIAM BOYD. Pp. 946, 1932, Philadelphia, Lea & Febiger, \$10.00.

The wide experience of the author as a teacher, has made him peculiarly fitted to write a text-book for students on the subject of Pathology. This text has been written from the standpoint of the student and is not intended to be an exhaustive reference book on the subject but the book is also intended for use by the clinician who wishes to brush up his knowledge of morbid anatomy. The text differs from the usual ones in pathology in that it presents the subject from the standpoint of physiology rather than from the standpoint of histology although this latter phase is by no means neglected. There is a definite attempt on the part of the author to coördinate his subject with general medicine and surgery and to that end he has included a description of the symptoms of diseases showing how they are related to the pathological processes. There has been a definite attempt to develop in the student who uses this text an attitude of mind which will cause him to seek correlation between pathology and symptoms.

The book is divided into general pathology and special pathology and instead of giving references, the author has given lists of related research for additional reading. The book is well written and in an extremely readable style. The illustrations, more than 275, are original and are excellently selected and reproduced. An unusually complete index makes the subject material immediately available to the casual reader.

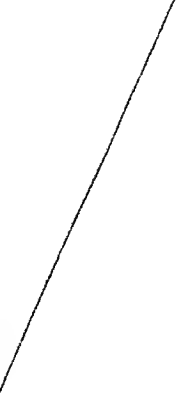
Oral Spirochetes and Related Organisms in Fusio-Spirochetal Diseases. BY DAVID T. SMITH. Pp. ix + 243, 1932, Baltimore, The Williams & Wilkins Company, \$4.50.

This is the first comprehensive work on the subject and the first available in the English language. The author takes the position that "While there is no evidence that oral spirochetes

alone can produce disease in man, they are probably the most important member in a symbiotic group of anaërobic organisms capable of initiating a severe and often fatal disease."

After learning from the introduction that the clinical lesions are rather characteristic, the diagnosis easily made and the treatment fundamentally the same, one wonders why a monograph of this length should be written, but the necessity of such a book is apparent by casually scanning the 822 references at the end of the book and by carefully reading the text. The author has given an excellent description of the organisms involved, their method of growth and demonstration, the pathology they produce both naturally and experimentally and the method of prevention and treatment. One learns that these organisms cause disease in almost every part of the body and that these lesions are by no means infrequently encountered in laboratory and clinical practice. To the general clinician to pathologists and the oral specialist the book will prove of tremendous value. Although it is evident that the subject is by no means closed, the author has revealed the state of knowledge up to the present time.

The Clay-Adams Company has specialized in hemacytometers and pipettes for many years and have advertised them in the JOURNAL since the first issue. These instruments are high class products and guaranteed to be accurate.



VIABILITY OF TUBERCLE BACILLI*

EFFECT OF MECHANICAL SHAKING AND CHEMICALS USED IN CONCENTRATION TECHNIC

JOSEPH E. POTTENGER

The Laboratory of the Pottenger Sanatorium, Monrovia, California

Inoculation of untreated materials into test animals has been the rule in the past, because of possible ill effects of chemicals and prolonged mechanical treatment on the viability of tubercle bacilli. It has been considered better to risk the loss of one or more animals by contaminating organisms than to face possible failure through lowering of virulence of the tubercle bacilli by previous treatment.

The main reason for chemical treatment is the elimination of contaminating organisms particularly for culture work and guinea-pig inoculation. By employing the mechanical shaker a further desirable result is attained in that the tubercle bacilli are scattered uniformly through the material to be examined, so that all portions of equal amount contain approximately the same number of organisms.

In the investigation of sputum, there is a growing tendency to use material saved for several days. The justification for such procedure is mathematically certain, but the disadvantage lies in two directions: the increase in contaminators and the possible ill effect of the aging of the specimen on the viability of the tubercle bacilli contained therein. The collection of a three-day specimen of sputum for microscopic examination has been routine practice in our institution for twenty-two years, from all patients in whom tubercle bacilli have previously not been found and also in those carrying rare tubercle bacilli. This method described and advocated elsewhere¹ applies to about one-half of all specimens ex-

* Read before the Eleventh Annual Convention of the American Society of Clinical Pathologists, New Orleans, Louisiana, May 6-9, 1932.

amined. No evidence has been noted of the disappearance of tubercle bacilli from these specimens, as some writers fear, but doubt has existed as to their viability.

A large literature is available dealing with virulence and pathogenicity of tubercle bacilli for guinea-pigs but as most of the work has been carried out with pure cultures, it is not reasonable to assume that conclusions, derived from that method of investigation are tenable, when working with tubercle bacilli from body secretions, from exudates, or from lesions in other inflammatory conditions. The environment is markedly different. Moreover, the dosage of tubercle bacilli in most experiments has been too high for comparison with dosage given to many of our animals, in practice. Working with an accurate counting method, it was shown² that the dilution-flotation-picric acid procedure would detect tubercle bacilli in a ten-minute search in sputum, when present to the number of only 70 to 400 per cubic centimeter of material, depending on the nature of the specimen. The use of the guinea-pig is materially restricted thereby.

Having been engaged for some time in checking this procedure by guinea-pig inoculation, it seemed highly desirable to determine the viability of the tubercle bacilli under the mechanical and chemical influences employed in practice. A further departure from accepted procedure was necessary in order to secure smooth films for counting, in that all dilutions were made with distilled water instead of with salt solution. The crystallization of salt disturbs seriously the uniformity of the film. The experiments to be described are an attempt to determine what effect, if any, these various treatments have upon the viability of the tubercle bacillus.

EXPERIMENTAL CONDITIONS

Six fresh purulent morning sputums were collected from a selected group of patients running a subacute course, in order that grouping of tubercle bacilli might be reduced to a minimum, and greater accuracy of count secured. Each sputum was diluted with equal parts of 0.6 per cent NaCl, shaken for thirty minutes in a strong shaker, and divided into three fractions.

Weighed portions of the first fractions, 1 cc. more or less, were diluted at least 1:30 with distilled water and shaken for twenty minutes, from which the number of tubercle bacilli per cubic centimeter was determined. The emulsions for counting were diluted further with distilled water and shaken ten minutes, in order to secure the desired number of tubercle bacilli per cubic centimeter for dosage. Other weighed portions, 1 cc. more or less, of the first fractions were treated with 0.5 per cent NaOH at 37°C. for one to three hours, diluted and shaken similarly for dosage.

The second fractions were set at 37°C. for fifteen to forty hours and shaken for ten minutes. Weighed portions, 1 cc. more or less, were treated with equal parts of 0.5 per cent NaOH for one to two hours, and other weighed portions with 3 per cent NaOH for twenty-five to thirty-five minutes and diluted exactly as in the first fractions for dosage.

The third fractions were allowed to stand at room temperature ranging from 15° to 28°C. for five to nine days and shaken for ten minutes. Weighed portions were treated with 0.5 per cent NaOH for one to four hours and 3 per cent NaOH for thirty to thirty-five minutes, diluted and shaken as above for dosage.

THE COUNTING METHOD

Precision slides, tested by micrometer, were selected so that a variation in thickness at the four corners and center did not exceed $\frac{1}{16}$ mm. These were placed on a plate glass platform which had been leveled by spirit level over a drying oven. The sputum diluted at least 1:30 with distilled water was shaken by mechanical shaker for thirty minutes. Exactly 1 cc. of this homogenized dilution was placed on the slide and allowed to dry. (If perfectly level the film dries symmetrically with respect to both axes of the slide in about fifteen minutes.) The slides were stained as usual, decolorized with 5 per cent H_2SO_4 , for one minute and immersed in water five minutes. The slides were covered with 10 per cent sodium sulphite to which had been added one-fifth volume of 95 per cent alcohol, until thoroughly bleached (five to ten minutes). The slides were immersed in water for five minutes so that all sulphite was removed. If the pink color returned another application of sulphite was made, followed by washing. Picric acid, 1 per cent, was used as a counterstain. The slide was placed on top of and perpendicular to a slide held by the mechanical stage. A small amount of cedar oil between the slides kept them in place. Every bacillus was counted from side to side 2 cm. from each end, using a limiting frame in the ocular.

The slide was 75,000 micra in length and the frame 89.5 micra in width. Therefore, $75000/89.5 = 838$, the factor determining the number of tubercle bacilli per cubic centimeter of diluted sputum from the average of the counts made.

It was found that unless a purulent sputum was diluted at least 1:30 the film would be too thick, causing some of the tubercle bacilli to be understained and likely to be overlooked in counting. The desirable dilution for the count yielded from 100 to 400 tubercle bacilli, from side to side, averaging about one to four bacilli per field. If the number exceeded this, a further dilution with shaking was made for the final count. Under these conditions maximum penetration of stain was attained in 95 per cent of all bacilli. The number of pale bacilli were counted and if they exceeded 5 per cent, the preparation was discarded. This standard was not difficult to attain, and failure of the first effort occurred but in one of the six specimens.

TABLE 1
COUNTS OF TUBERCLE BACILLI BY THE PRECISION SLIDE METHOD

| SPUTUM | NUMBER OF TUBERCLE BACILLI COUNTED | | | | MEAN | PROBABLE ERROR OF THE MEAN |
|--------|------------------------------------|-----|-----|-----|------|----------------------------------|
| | A | B | C | D | | |
| 1 | 93 | 116 | 111 | 166 | 121 | per cent ±8.6 |
| 2 | 156 | 179 | 191 | | 175 | ±3.9 |
| 3 | 250 | 303 | 274 | 239 | 266 | ±3.5 |
| 4 | 447 | 485 | | | 466 | ±2.8 |
| 5 | 435 | 452 | | | 443 | ±3.3 |
| 6 | 140 | 146 | | | 143 | ±1.4 |

A few counts were made on the second and third fractions. The results were usually within the probable error of the counting method, and no evidence of growth was found. The count of the second fraction, which had been set at 37°C. for fifteen to forty hours, was usually less than that obtained on the first fraction, and some of the tubercle bacilli found took the stain poorly as has been repeatedly pointed out by other workers. The number of pale bacilli exceeded 5 per cent and were found only by proceeding very slowly.

If the first two counts did not vary more than 15 per cent of the lesser, the result was considered satisfactory; if greater than 15 per cent, additional counts were made. The probable error in a 15 per cent variation is ±5.1 per cent.

Table 1 gives the detailed counts with the probable errors computed. With the exception of the counts for sputum 1, the probable errors are about the same as in careful leukocyte counting. The time required to make a count across the slide averaged about ten minutes.

ANIMAL EXPERIMENTS

Table 2 gives the detailed record of treatment and dosage of tubercle bacilli, and the results, at autopsy, in guinea-pigs.

All guinea-pigs, with the exception of one, showed widespread tuberculosis usually with caseation in one or more of the mesenteric nodes and spleen, less frequently in the liver, pancreas, bronchial glands, and lungs. Those inoculated intraperitoneally, showed involvement frequently in bronchial lymphatic nodes and

TABLE 2

SHOWING RESULTS OF GUINEA-PIG INOCULATION WITH SPUTUM

Shaking mechanically with and without previous treatment with sodium hydrate, 0.5 per cent and 3 per cent. Guinea pigs killed from 56 to 61 days after inoculation.

| METHOD OF INJECT- ING GUINEA-PIGS | DOSAGE OF BACILLI | FRACTION 1 FRESH SPUTUM | | | FRACTION 2 SPUTUM AT 37°C. | | | | | FRACTION 3 SPUTUM AT 12°-23°C. | | | | |
|--------------------------------------|-------------------|----------------------------|-----------------|--------|-------------------------------|-------------------|--------|---------------|--------|-----------------------------------|-------------------|--------|---------------|--------|
| | | Treated with | | | Interval before treatment | Treated with NaOH | | | | Interval before treatment | Treated with NaOH | | | |
| | | H ₂ O | | NaOH | | | | | | | | | | |
| | | Result | 0.5 per cent | Result | | 0.5 per cent | Result | 3 per cent | Result | | 0.5 per cent | Result | 3 per cent | Result |
| | | | hours | | | hours | hours | | min. | | | days | hours | |
| Left axilla..... | 200 | ++ | | | | | | | | 9 | 1 | ++ | | |
| Left axilla..... | 300 | ++ | 3 | ++ | 24 | | | 35 | ++ | 6 | 4 | ++ | 30 | + |
| Left axilla..... | 200 | ++ | 1 | ++ | 17 | 2 | ++ | 25 | ++ | 5 | 2 | ++ | 35 | ++ |
| Intraperitoneal. | 100 | | 2 | ++ | 40 | 2 | ++ | 30 | ++ | 5 | 1 | ++ | | |
| Intraperitoneal. | 100 | | 1 | ++ | 23 | 2 | ++ | 25 | ++ | | | | | |
| Intraperitoneal. | 50 | ++ | 3 | ++ | 15 | 1 | ++ | 25 | ++ | 5 | 2 | ++ | 30 | ++ |

+ = tuberculosis of small degree.

++ = wide spread tuberculosis.

lung. In the one exception yielding an unsatisfactory result, the inoculum treated with 3 per cent NaOH was injected in the axilla. The organisms were recovered from the inoculation site and axillary nodes but all other organs appeared normal and no tubercle bacilli could be demonstrated in them.

Tubercle bacilli were searched for in preparations made from the caseous material or crushed organs after thorough grinding first without, then with 0.5 per cent NaOH. In some organs in

which bacilli were not found or were rare, the digested material was treated by the dilution-flotation procedure. Because of the high protein content it was necessary to carry out the dilution feature much farther than is necessary in sputum. Approximately 1 gram of digested organ was diluted to 500 cc. with distilled water before shaking with 0.5 cc. of xylol. Erlenmyer flasks were used for the purpose. Usually suspected lesions were small, weighing but a few milligrams, and were removed,

TABLE 3
EXAMINATION OF ORGANS OF INJECTED GUINEA-PIGS

A comparison of direct smear and dilution-flotation method with records of numbers of tubercle bacilli found in the organs of animals and number of minutes spent in search.

| ORGAN | METHOD | | | | ORGAN | METHOD | | | |
|-----------------|--------------|----------|--------------------|----------|------------------|--------------|----------|--------------------|----------|
| | Direct smear | | Dilution-Flotation | | | Direct smear | | Dilution-Flotation | |
| | Bacilli | Min-utes | Bacilli | Min-utes | | Bacilli | Min-utes | Bacilli | Min-utes |
| Spleen | 0 | 20 | 10 | 5 | Mesenteric nodes | 40 | 5 | 50 | 5 |
| | 2 | 20 | 45 | 5 | | 1 | 20 | 10 | 5 |
| | 0 | 20 | 15 | 5 | | 6 | 5 | 83 | 5 |
| | 3 | 5 | 63 | 5 | | 3 | 10 | 23 | 5 |
| | 3 | 10 | 13 | 5 | | 0 | 10 | 2 | 15 |
| | 4 | 5 | 255 | 5 | | 3 | 1 | 75 | 10 |
| | 0 | 5 | 3 | 5 | | 0 | 10 | 10 | 2 |
| Liver | 1 | 20 | 8 | 5 | Axillary nodes | 3 | 5 | 46 | 5 |
| | 0 | 10 | 1 | 10 | | | | | |
| Lung | 0 | 20 | 1 | 10 | | | | | |
| Bronchial nodes | 0 | 5 | 3 | 5 | Injected area | 14 | 5 | 154 | 5 |
| | 8 | 5 | 24 | 5 | | | | | |

ground and digested for thirty minutes, then treated in a test tube with 5 to 10 cc. of water and 4 to 6 drops of xylol for the dilution-flotation procedure.

Table 3 gives a few comparisons of results obtained by the direct smear and dilution-flotation method, showing the marked superiority of the latter.

DISCUSSION

Adequate control against spontaneous infection in these animals is found in the fact that concurrently with the experimental

work forty-three guinea-pigs of the same stock were routinely inoculated as a check on the dilution-flotation procedure: in thirty-two instances both tests were negative; in four, both were positive; in five, the dilution-flotation was positive and guinea-pig negative; and in two, the dilution-flotation was negative and guinea-pig positive.

The experimental conditions under which this work was carried on presents two features wholly at variance with the customary procedure: first, the use of a strong shaking machine instead of grinding in a mortar to secure uniform distribution of tubercle bacilli; second, the substitution of distilled water for saline solution as a diluent, for technical reasons. Willis'⁴ recent work in showing the gradual loss of pathogenicity of dry tubercle bacilli ground in a ball mill suggests that grinding as usually employed may not be wholly free from effect on the viability of the tubercle bacilli. The grinding of dry bacilli would no doubt result in much greater damage than when carried out under the usual moist conditions.

Strong evidence has recently been given³ that tubercle bacilli in sputum are injured by contact with 3 per cent NaOH for thirty minutes. On the other hand much stronger solutions have been employed as digestants, after which viability was demonstrated by guinea-pig inoculation. One wonders in the latter instance whether the homogenization was adequate to assure actual contact with the chemical. The action of 0.5 per cent NaOH on the tubercle bacillus seems not to have been determined. It is a complete solvent of pus and cells composing organs, though it acts more slowly than stronger solutions. As a digestant it seems to accomplish all that can be accomplished by stronger solutions, and its effect on the viability of the tubercle bacillus, if any, should be less. It does not kill all contaminators in sputum but does inhibit their influence in determining mortality in guinea-pigs.

Of a total of 110 animals inoculated from material of diverse origin, after treatment by 0.5 per cent NaOH, only two died from subcutaneous and two from intraperitoneal inoculation within three weeks. These four deaths resulted from inoculation of sputum which had been in contact with 0.5 per cent NaOH for

one to three hours. The maximal time of contact with the digestant without producing injury to the tubercle bacilli is not known, but is being investigated. To date successful inoculation has followed contact in individual instances for forty, thirty-six, thirty, twenty-two and sixteen hours. Such results suggest the possibility that prolonged contact may eliminate the mortality due to contaminants without doing injury to the tubercle bacilli.

SUMMARY AND CONCLUSIONS

Six different sputums were homogenized by mechanical shaker and divided into three fractions: the first fractions were used fresh; the second stood at 37°C. for fifteen to forty hours; and the third stood at room temperature 15° to 28°C. for five to nine days. Portions of the three fractions were treated with 0.5 per cent NaOH for a period of one to four hours. Portions of the second and third fractions were treated with 3 per cent NaOH for twenty-five to thirty-five minutes. A dosage of fifty to 300 tubercle bacilli following each of these treatments resulted in widespread tuberculosis in twenty-five of a total of twenty-six guinea pigs in fifty-six to sixty-one days. One guinea pig, inoculated with 300 tubercle bacilli from a fraction which stood at air temperature for six days, followed by treatment with 3 per cent NaOH for thirty-five minutes, did not develop widespread disease.

Dilution with distilled water, followed by mechanical shaking for one hour, apparently caused no injury to tubercle bacilli found in fresh sputum.

Similar treatment of fresh, autolyzed (37°C. for fifteen to forty hours) and old specimens (six to nine days) of sputum, after previous digestion of the specimens with 0.5 per cent NaOH for one to four hours, apparently caused no injury to the tubercle bacilli, but injury is probable when 3 per cent NaOH is used as a digestant, for twenty-five to thirty-five minutes.

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A NEW AND SIMPLIFIED MEDIUM FOR PASTEURELLA TULARENSIS AND OTHER DELICATE ORGANISMS

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Since the discovery of *Pasteurella tularensis* (*Bacterium tularensis*) by McCoy and Chapin³ the widespread interest in tularemia which followed the accurate work of Francis^{1,2} and of Simpson^{5,6} has made it imperative that clinical laboratories be equipped to make agglutination tests for this disease commonly known as "rabbit fever."

In practice, the technical difficulties attending the preparation of suitable fresh media and keeping alive cultures of *P. tularensis* to furnish sufficient antigen supply are such that the small laboratory finds difficulty in keeping prepared for the infrequent calls for this test. The organism was cultivated by McCoy and Chapin on Dorsett egg medium, on which a scant transparent growth occurred in five days, by Wherry and Lamb⁸ on coagulated egg yolk, by Francis on horse or rabbit serum-glucose agar enriched by a bit of healthy rabbit spleen, and on Loeffler's blood serum coagulated at 20°C. On all of these media the growth appeared in four to six days as minute transparent droplets resembling the white of egg. Francis has since adopted fresh blood-glucose-cystine agar as the most satisfactory medium for *P. tularensis*. This contains 1 per cent peptone, 1.5 per cent agar, 0.5 per cent sodium chloride and is adjusted to a pH of 7.3. When needed there is added to the stock agar 0.1 per cent amino-acid in the form of cystine and 1 per cent glucose; this is heated in an Arnold sterilizer sufficiently long to melt the agar, and to dissolve and sterilize the cystine and glucose. The mixture is then cooled to 40 to 45°C. when 5.0 to 8.0 per cent of defibrinated rabbit blood is added, after which it is tubed, slanted and incu-

bated to test sterility. Precaution must be taken against overheating with loss of the bright red color. Cystine is not readily soluble so that in the Francis medium some of it remains undissolved. Shaw⁴ attempted complete solution by placing the mixture in a water bath at 100°C. or in an autoclave at 15 pounds pressure for fifteen minutes. Transplants were made on this medium every two months from stock cultures kept in the ice box.

In my experience there is considerable hazard in preparing blood-glucose-cystine agar owing to technical difficulties, particularly in regard to the collection, manipulation and addition of sterile rabbit blood subsequent to sterilization of the medium, and difficulty of dissolving cystine, and finally the uncertainties as to the sterility of the media, for contaminations will show up later to spoil the cultures, that were not brought out by the test incubation. Thus, cultures died out or were contaminated on sample medium made by myself and by two biological supply houses.

Since there is no available source from which antigen can be obtained, an effort was made to overcome these difficulties. Following the suggestion of Spray⁷ on the use of heated blood derivatives for cultivation of hemoglobinophilic organisms, the idea presented itself of using dehydrated hemoglobin instead of fresh defibrinated rabbit blood. This would make the medium easier to prepare and safer to use since all ingredients could be sterilized. Thus, having a stock dehydrated cystine agar and stock dehydrated hemoglobin any laboratory could make fresh medium for *P. tularensis* as needed.

Collaborating with the Difco Laboratories, Detroit, Michigan such a fool proof medium has been evolved and has so far proved entirely satisfactory. This medium is not offered as being superior to blood-glucose-cystine agar, but rather a dehydrated, prepared stock medium that can be autoclaved and with which any laboratory can routinely prepare such amounts of medium as meet their requirements. This medium gives a luxuriant growth of *P. tularensis* within three to four days, of cream gray color, furnishing ample material for making antigen. The new medium is made by mixing Spray's Bacto hemoglobin in equal parts, with

Bacto cystine heart agar, which latter is essentially a modified Huntoon hormone agar.

*Formula of dehydrated "Bacto Cystine Heart Agar" (Difco)**

| | |
|-------------------------------|---------|
| Beef heart Infusion from..... | 500 gm. |
| Bacto peptone..... | 10 gm. |
| Bacto dextrose..... | 10 gm. |
| Sodium chloride..... | 5 gm. |
| L-cystine..... | 1 gm. |
| Bacto agar..... | 15 gm. |

Reaction: pH 6.8. (The reaction may range between 6.8 and 7.3 but in my hands 6.8 gave the most luxuriant growth.)

Both the Bacto cystine heart agar and Bacto hemoglobin can be obtained in dehydrated form as a stock supply.

Method for preparing 500 cc.

(A) "Double strength agar"—Dissolve, by boiling 28 grams Bacto cystine heart agar in 250 cc. distilled water. Sterilize for twenty minutes at 15 pounds pressure (250°F.). The sterile agar will be dark in color (chocolate agar).

(B) Dissolve 5 grams Bacto hemoglobin in 250 cc. distilled water and strain through gauze to remove any large undissolved particles. Sterilize twenty minutes at 15 pounds pressure.

(C) Cool both of the above sterile solutions (A and B) to 50 to 60 C. and mix. Dispense in sterile test tubes or other containers. Use strictly aseptic conditions. Incubate to test sterility.

Besides its special value as *P. tularensis* medium* and on account of its very high nutritional qualities, which make it sensitive, this medium is also suggested for routine use as a general purpose medium, especially for cultivation of the various difficulty grown organisms such as the gonococcus, *Hemophilus influenzae* and *pertussis*, the meningococcus, pneumococcus and streptococcus. The luxuriant growth of most organisms makes it valuable for autogenous vaccines or antigens of any kind. It is particularly useful in growing pneumococci and streptococci, and

* At my request Dr. Walter M. Simpson tested the dehydrated cystine heart agar against his own medium. He reported that one sample with pH 7.3 gave fairly satisfactory growth of *P. tularensis* but that the second sample pH 6.8 yielded a very luxuriant growth. He concluded that this formula makes a most satisfactory medium for the cultivation of *P. tularensis*.

in studying their hemoglobinophilic properties, since it gives broad zones of decolorization, changing from dark chocolate to eight brown.

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A STUDY OF PATHOGEN-SELECTIVE CULTURES IN RELATION TO VACCINE THERAPY*

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The Solis-Cohen pathogen-selective method for preparing autogenous vaccines is based upon the assumption that organisms capable of growing in the fresh, whole, coagulable blood of the patient are those which are most pathogenic for that individual. This assumption followed the discovery that the whole blood of animals naturally resistant or immune to pneumococci¹ and to meningococci² is bactericidal for such organisms, while the blood of animals naturally susceptible to them lacks this bactericidal property. One of the advantages of the method is that it furnishes a means for selecting the etiologically important organisms from a mixed culture. The pathogen-selective method is not applicable to spore-forming organisms, to those producing exotoxins, or to those difficult to cultivate, like the gonococcus, *Hemophilus influenzae*, et cetera.

The method consists of two simultaneous inoculations of the material to be cultured, one in a rich medium, such as Rosenow's brain broth, and the other in the patient's fresh, whole, coagulable blood in vitro.^{5, 6, 7, 8} After a primary incubation of twenty-four hours, both cultures are examined and the organisms present in each are studied for identification. The organisms which appear in the blood are those elected to predominate in the vaccine.

The present study consists of 404 pathogen-selective cultures from 150 patients. The Solis-Cohen technic, described in detail by Kolmer and Boerner,² was used. The majority of the cultures

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were from the noses and throats of patients with subacute and chronic upper respiratory infections. Material from the rhinopharynx and from the tonsils, or tonsillar fossae, were often mixed and cultured together. The source and number of cultures are listed in table 1.

TABLE 1
SOURCES OF 404 PATHOGEN-SELECTIVE CULTURES FROM 150 PATIENTS

| SOURCE | NUMBER |
|--|--------|
| Nares..... | 163 |
| Nasal accessory sinuses..... | 9 |
| Tonsillar fossae and rhinopharynx..... | 108 |
| Tonsils and rhinopharynx..... | 58 |
| Tonsillar fossae..... | 1 |
| Tonsils..... | 5 |
| Rhinopharynx..... | 5 |
| Teeth..... | 15 |
| Sputum..... | 19 |
| Ear..... | 3 |
| Abscess..... | 5 |
| Urine..... | 4 |
| Feces..... | 6 |
| Anal fissure..... | 1 |
| Prostatic secretion..... | 1 |
| Carcinomatous ulcer..... | 1 |

TABLE 2
GROUPING OF CASES ACCORDING TO RESULTS OF PATHOGEN-SELECTIVE CULTURES

| GROUP | NUMBER |
|---|-------------|
| 1. Same organisms in broth culture and in patient's blood.. | 146 (36.1%) |
| 2. Several organisms in broth culture but not all growing in patient's blood..... | 108 (26.7%) |
| 3. Organisms in broth culture but none growing in patient's blood..... | 104 (25.7%) |
| 4. Organisms growing in patient's blood but none in broth culture..... | 46 (11.5%) |

All of the cases studied have been classified, according to the results obtained, into four groups as shown in table 2. In approximately one-third of the cases the results were identical in both the blood and the broth, as shown in group 1. In the case

of group 2, which constituted about one-fourth of the cases, only certain of the organisms present in the broth grew in the blood. These were selected to predominate in the vaccines. The results in group 3 suggest the possibility that the organisms of etiological importance were not present in the material cultured or had been missed due to error in technic. The tests in these cases were repeated and if the same result was obtained, other foci were looked

TABLE 3
ORGANISMS PRESENT IN 404 PATHOGEN-SELECTIVE CULTURES FROM 150 PATIENTS

| ORGANISM | NUMBER OF STRAINS ISOLATED | NUMBER AND PERCENTAGE WHICH GREW IN PATIENT'S BLOOD | NUMBER AND PERCENTAGE WHICH DID NOT GROW IN PATIENT'S BLOOD |
|--|----------------------------|---|---|
| | | % | % |
| Streptococci (hemolytic)..... | 32 | 25 (78.1%) | 7 (21.8%) |
| Streptococci (viridans group)..... | 63 | 41 (65.0%) | 22 (34.9%) |
| Streptococci (non hemolytic)..... | 160 | 98 (61.2%) | 62 (38.7%) |
| <i>Staphylococcus aureus</i> | 113 | 82 (72.5%) | 31 (27.4%) |
| <i>Staphylococcus albus</i> | 182 | 64 (35.1%) | 118 (64.8%) |
| <i>Staphylococcus pharyngis</i> | 3 | 1 (33.3%) | 2 (66.6%) |
| Micrococci (unidentified)..... | 8 | 2 (25.0%) | 6 (75.0%) |
| <i>Neisseria catarrhalis</i> | 15 | 0 | 15 (100%) |
| <i>Neisseria siccus</i> | 21 | 0 | 21 (100%) |
| <i>Neisseria perflava</i> | 1 | 0 | 1 (100%) |
| Gram negative cocci (unidentified)... | 19 | 4 (21.0%) | 15 (78.9%) |
| <i>Diplococcus pneumoniae</i> | 22 | 9 (40.9%) | 13 (59.0%) |
| <i>Corynebacterium pseudodiphthericum</i> .. | 21 | 9 (42.8%) | 12 (57.1%) |
| <i>Klebsiella pneumoniae</i> | 8 | 4 (50%) | 4 (50.0%) |
| <i>Escherichia coli</i> | 9 | 4 (44.4%) | 5 (55.5%) |
| <i>Proteus vulgaris</i> | 3 | 3 (100%) | 0 |
| Gram negative bacilli (unidentified)... | 21 | 2 (9.5%) | 19 (90.4%) |
| <i>Hemophilus influenzae</i> | 3 | 0 | 3 (100%) |
| <i>B. subtilis</i> | 8 | 2 (25.0%) | 6 (75.0%) |
| Yeasts..... | 3 | 1 (33.3%) | 2 (66.6%) |

for and cultured, especially if evidence of systemic infection existed. Group 4 shows that in 11.5 per cent of the cases organisms appeared in the blood, which would have been missed had only broth cultures been made.

The various organisms isolated and the frequency with which they appeared in the blood and in the broth cultures are listed in table 3.

A study of this table shows that organisms usually classed as pathogenic for man were the ones which grew most frequently in the patient's whole coagulable blood, namely streptococci and staphylococci. It is to be noted that the hemolytic strains of streptococci showed the highest percentage growing in the patient's blood and that the staphylococcus aureus grew more frequently in the blood than did other members of this group. The opposite was true of the Gram-negative cocci, which are slightly, if at all, pathogenic. Of these but four of the fifty-six cultures grew in the patient's blood. The pneumococcus grew in the blood nine times and failed to grow thirteen times. These strains were not typed but, being from non-contact cases, they probably belonged to group IV.

It is difficult to explain why such a high percentage of the diphtheroids grew in the patient's blood. Fifty per cent of Friedländer's pneumobacillus and four of the nine strains of *Esch. coli* (*B. coli*) grew in the blood. The occurrence of *B. subtilis* in the blood was no doubt due to the presence of resistant spores in the material cultured, which remained viable in the blood and grew upon subculturing. The unidentified Gram-negative bacilli were only studied sufficiently to prove that they were not of the pathogenic types. These were considered saprophytic and, as shown in the table, only two of the twenty-one strains grew in the patient's blood. The other organisms appeared too infrequent to permit of deductions or conclusions.

DISCUSSION

The data in this series of pathogen-selective cultures are very similar to those reported by Lowe.³ He reported upon 600 pathogen-selective cultures and classified his patients into five groups. His group of cultures giving similar results, which corresponds to our group 1, contained only 6 per cent. This difference may be due to the source of the cultures and to the types of the infections studied. Lowe made the statement that although apparently non-pathogenic organisms were present in a large number of cases; only in one of the thirty-four examinations did such an organism as *Neisseria catarrhalis* (*Micrococcus catarrhalis*) appear

in the blood-controlled culture. Our results as a whole are very similar to those of Lowe.

The question arises as to whether the pathogenic organisms which failed to grow in the patient's blood should be considered as entirely harmless or as capable of causing local irritation and inflammation, but not systemic infection. In this connection we have also to consider the possibility of bacteria producing systemic diseases by the absorption of their toxins from areas of local infection. Lowe was of the opinion that these organisms are capable of causing local but not systemic infection. These questions are debatable and with the present knowledge can not be definitely answered. In view of this fact we have not entirely ignored the pathogenic bacteria which failed to grow in the blood, but have included them in the vaccines to the extent of 10 per cent. The following is the manner in which the organisms were selected for vaccines:

In Group 1 the organisms growing in the blood rather than those growing in the broth were selected for the vaccine, even though they appeared identical.

In Group 2 90 per cent of the organisms which grew in the blood and 10 per cent of the pathogens which grew in the broth, but failed to grow in the blood, were included in the vaccine. Organisms considered as non-pathogenic, which grew in the broth only, as well as spore-bearers were not included.

In some cases of Group 3 vaccines were prepared from the broth cultures for the purpose of combating only the local infection. Where evidence of systemic infection was present other foci of infection were looked for and studied.

Group 4 was considered the same as group 1.

The clinical results of the vaccines prepared from the cultures reported in this paper will be the subject of a future publication by one of us (M. S-C).

The pathogen-selective method of culturing has also been found useful by one of us (F. B.) as an aid in isolating such organisms as the streptococci from mixed infections and contaminated material. The patient's fresh, whole, coagulable blood in such cases very often retards or inhibits the growth of the unimportant

organisms which overgrow in the ordinary culture. As an example, a nasal culture received for the preparation of a vaccine, was overgrown with *Proteus vulgaris* but in which streptococci were recognized in smears. The culturing was repeated upon two occasions with the same results. It was then decided to inoculate the nasal secretions into the patient's blood. This was done, with the result that streptococci and staphylococci were isolated without interference with *P. vulgaris* as the blood proved bactericidal for this organism and thus eliminated it from the culture.

CONCLUSIONS

(1) A series of 404 pathogen-selective cultures from 150 patients were studied.

(2) In many cases the isolation of the more important pathogenic organisms was aided by the bactericidal action of the patient's fresh, whole, coagulable blood upon the less important and presumably non-pathogenic organisms.

(3) Information was obtained regarding the presence or absence of bactericidal substances in the patient's blood for the various organisms isolated.

(4) In about 11 per cent of the cases organisms were isolated which would have been missed by the ordinary methods of culturing.

(5) A basis is provided for selecting the organisms to pre-dominate in an autogenous vaccine, on the assumption that the organisms which grow in the patient's fresh, whole, coagulable blood are of most importance to the individual.

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A STUDY OF O AND H AGGLUTININS IN TYPHOID AND ENDEMIC TYPHUS FEVER*

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The applications of the work of Felix³ in qualitative receptor analysis to problems of serum diagnosis in the enteric fevers, particularly typhoid, has so far engaged the attention of English and continental investigators that today they hold rather definite conceptions of the situation in regard to the serum diagnosis of typhoid, particularly in the inoculated individual. The finding of H, or flagellar antibodies, in the serum of previously inoculated individuals has been so constant since the first reports by Felix; Topley, Platts and Imrie; Rosher and Fielden, and MacVie and Smith, "That we cannot assess the significance of a positive agglutination test with any certainty if we know that the patient has received a previous prophylactic inoculation, or if we are uncertain on that point."⁵ Topley and Wilson⁵ offer two ways of escape from this apparent dilemma: The application of the principles of qualitative receptor analysis (Felix), and serial quantitative estimations, the so-called comparative Widal test of Dreyer. The encouraging results with the qualitative methods published by the English workers mentioned above, and the more recent reports by American investigators, Eldering and Larkum² led to this study.

In regard to the H agglutination, this study brings out no essential differences in the now well established mechanism governing this phenomenon (Craigie¹). As has been observed many times before, it was noted that the serum of typhoid vaccinated individuals, in typhoid cases, in endemic typhus cases and in cases of undulant

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fever agglutinated (sometimes at high dilution) the 0.1 per cent formalized broth cultures of typhoid bacilli accepted as H antigen. Our main interest, therefore, became centered upon the problem of O agglutination as applied by Felix to the diagnosis of typhoid in the some time inoculated cases. Felix^{3,4} strongly contended that the individuals inoculated with typhoid-paratyphoid vaccine do not develop O agglutinins for typhoid organisms which have been selected in their O phase of growth. Instead of the phase-selected organisms the use of mechanically deflagellated bacteria, or alcoholized bacteria, has been so successful in the hands of so many English investigators that the Topley and Wilson text accepts the alcoholized typhoid bacilli as O antigen as fully as the formalized broth cultures for H antigen. It appears that such an O antigen may be prepared by treating thick emulsions of typhoid bacilli with 50 per cent alcohol and subsequently diluting this antigen to the desired turbidity. It is necessary that the culture so treated be definitely S type organisms since S→R dissociation brings about a change in the specific somatic (O) substance to a non-specific agglutinin (Ø) found to be shared by unrelated species of bacteria. Alcohol may definitely affect the reaction, but it has been found that a final concentration of as much as a 5 per cent alcohol in the tests has no effect upon the reaction.

TECHNIC

The following O antigens were used: a 50 per cent alcoholized emulsion diluted to standard turbidity, final alcohol concentration 2.5 per cent; a 10 per cent alcoholized eighteen hour broth culture, final alcohol concentration 2.5 per cent; a culture of growth phase O antigen (Felix's Ty O-901 obtained through the courtesy of Dr. N. W. Larkum, who obtained it from Drs. W. W. C. Topley and A. D. Gardner of the British Medical Research Council) and the same culture, Ty O-901, treated with 0.2 per cent formol.

The idea in using the 10 per cent alcoholized antigen was to find out if the broth cultures so treated might be usable and so make possible the elimination of the steps necessary to preparing thick emulsions.

With regard to the formalizing of the O growth phase culture, it is to be noted that despite the fact that Topley and Wilson commented rather frequently in their text on the depressing activity of formalin on O agglutination, and in spite of the fact that Felix⁴ had shown definitely that formalin does depress the activity of the somatic agglutinin, the results of Eldering and Larkum² tended to

show that the presence of 0.1 per cent formol in the Ty O-901 culture does not sufficiently depress the agglutination of this selected O antigen to the extent that there is definite interference with agglutination.

The sera employed in this study came from four groups of individuals. The first was a group of six cases of proven typhoid fever, three of whom had had, three to five years previously, typhoid-paratyphoid vaccine, and three who had not had typhoid-paratyphoid vaccine. It was possible to follow their serum reactions throughout the course of their illnesses. A second group was composed of fifteen cases of typhoid in which at least two samples of sera were obtainable during the course of their illnesses. The third and fourth groups were, respectively, thirty typhoid-paratyphoid vaccinated Reserve Officers Training Corps students, and thirty individuals whose sera were obtained for another problem, a study in chronic arthritis.

In carrying out the tests equal parts of antigen and serum (1 cc.) were incubated at 37 degrees centigrade for eighteen hours, following which the tests were placed in the ice-box for two hours before reading.

A SERIAL STUDY OF SIX TYPHOID CASES

In the study of the six typhoid cases, serum taken within the first week failed to agglutinate any of the O antigens. In the second week it was found that both alcoholized antigens were agglutinated in all six cases at the following low dilutions: 1:20, 1:20, 1:40, 1:20, 1:60, and 1:80. The growth-phase antigen, Ty O-901, and the formolized antigen were not agglutinated at this time, nor during the next, the third week. During the third week the agglutinating titers for the alcoholized antigens rose perceptibly, becoming 1:80, 1:160, 1:160, 1:160, 1:240, 1:340, in the order first named.

A 0.1 per cent formolized broth culture of *Eberthella typhi* used as a sort of control in these tests was agglutinated for the first time (the third week) in serum from cases 4, 5, and 6, in dilutions of 1:40, 1:80, and 1:60 respectively. In the fourth week the agglutinating titers for the alcoholized antigens reached the following high levels: 1:320, 1:640, 1:480, 1:640, and 1:640. The growth phase O antigen was agglutinated at some dilution by all of the sera during the fourth week. The titers were, however, very low compared to the high titers with the alcoholized antigens. They were 1:40, 1:40, 1:20, 1:30, 1:60, and 1:40 respectively. The formolized growth-phase culture was agglutinated by one

serum only, number 6, at a dilution of 1:40. The formolized culture of *E. typhi* was not agglutinated by the sera of cases 1, 2, and 4, while the sera of cases 3, 5, and 6, increased in agglutinating

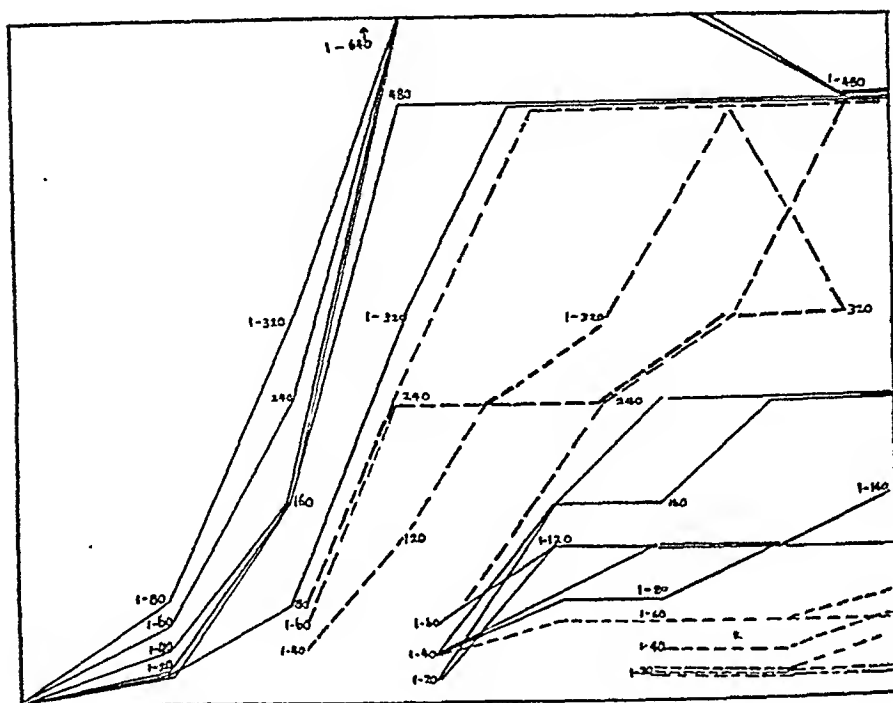


FIG. 1. AGGLUTINATING TITERS IN SIX CASES OF TYPHOID FEVER STUDIED DURING A PERIOD OF EIGHT WEEKS

(1) The unbroken lines at the left show the agglutinating titers of the patients' sera with alcoholized (artificial O) agglutinin. (2) The broken lines in the center show the agglutinating titers with 0.1 per cent formolized *E. typhi*. (3) The unbroken lines in the lower right represent the agglutinating titers with Felix's Ty O-901 (growth-phase selected O agglutinin). (4) The broken lines in the lower right represent the agglutinating titers with 0.1 per cent formolized Ty O-901. (5) The numerals (e.g. 1:320) represent the dilutions of the patients' sera found to agglutinate the various antigens at weekly intervals beginning with the second week of the illness.

titer for this antigen to 1:120, 1:240, 1:340 respectively. During the fifth week the alcoholized antigens were still agglutinated at relatively the same levels. The growth phase O antigen was

agglutinated at increasingly higher levels: 1:80, 1:80, 1:120, 1:160, 1:120, 1:160, and the formolized growth phase O antigen was agglutinated by two sera, numbers 4 and 6, at dilutions of 1:60 and 1:60 respectively. The formolized culture of *E. typhi* was agglutinated by four sera, 3, 4, 5, and 6, at dilutions of 1:240, 1:80, 1:240, and 1:480, respectively. Serum from cases 1 and 2 still failed to agglutinate the formolized culture of *E. typhi*.

In the sixth week there were no essential changes in the agglutinating titers except in the case of the formolized growth phase O antigen wherein some agglutination was noted in each case, but all of these were at a relatively low titer, namely 1:20, 1:20, 1:20, 1:60, 1:40, and 1:60. Cases 1 and 2 were still negative to the formolized broth culture of *E. typhi*. Cases 1, 2, 3, and 4 were making fair progress toward recovery and cases 5 and 6 were nearing convalescence.

In the seventh week after the onset of the disease, serum was again tested in these six cases. No essential changes from the sixth week were found.

In the eighth week serum obtained from the six patients, four of whom were definitely convalescent, the other two nearly so, showed but little change from the sixth and seventh week. Agglutinating titers for the formolized growth-phase cultures were slightly higher on the whole, and the serum of case number 2, which had been persistently Widal negative throughout the course of the illness, at this time partially agglutinated the formolized broth culture of *E. typhi* at a dilution of 1:40.

In this series cases 1, 2, and 3 were individuals who had never been vaccinated against typhoid and paratyphoid and who also gave no history of similar previous illnesses. Cases 4, 5, and 6 had had either full or partial vaccination at intervals varying between three to seven years prior to the onset of their illness. A brief history of their vaccination follows. Case 4 had had two doses of typhoid-paratyphoid vaccine three years prior to onset of present illness. Case 5 had had a full course of vaccine seven years previously. Case 6 had had one dose of vaccine a year and a half before the onset of the present illness.

All of these patients recovered. There were no serious compli-

cations in any of the cases studied. Convalescence in case 2 was prolonged some three weeks longer than in the others. Clinically, then, there were no very great differences in these cases. Serologically, the previously vaccinated group showed the presence of agglutinins sooner and at higher titer than the non-vaccinated group. In this connection the point to be emphasized particularly is the fact that the agglutination of the growth phase O antigen appeared later and at lower titers than the alcoholized or formolized typhoid antigens. The formolized growth-phase antigen was agglutinated only after a longer period of time and at relatively lower titers than even the growth phase O antigen. Typhoid-paratyphoid vaccination, apparently, had some effect upon the reactions. However, O agglutinins were found at practically the same time in the serum of both the previously vaccinated and the non-vaccinated.

The complete failure of one serum, and the partial failure of another, to agglutinate the H antigen is nothing new in the experience of individuals who have done many Widal tests in cases clinically typhoid. Felix⁴ reported 27 per cent of 531 proved cases of typhoid reacting negatively to Widal tests in which formolized typhoid cultures were used as antigen. In all of these cases positive agglutination of O antigen was observed. Felix also quotes Pijper as recording 28 per cent of 120 proved cases of typhoid in South Africa, which were persistently "Widal negative" to 0.1 per cent formolized broth cultures.

It appears, then, that somatic (O) antibodies are produced at some time during typhoid fever, however, in low titer, and that any attempt at serum diagnosis of typhoid which fails to include an antigen for their detection may fail because of the absence of H agglutinin in a rather high percentage of cases.

AGGLUTININS IN FIFTEEN TYPHOID CASES

In the second group of cases studied, fifteen in all, serum was obtainable only twice during the illness, the first samples being obtained within the second ten-day period of the disease, and the second samples being obtained within the third and fourth ten-day periods of the illness. Ordinarily we expect the Widal to

become positive at some time within the second ten-day period. Certainly in proved cases of typhoid we expect a high percentage of positive reactions by the time the third and fourth ten day periods have been reached.

Without detailing here the data in the fifteen cases in this group, but from taking the findings by and large, it appears that alcoholized antigens are agglutinated sooner and at remarkably higher titers than the growth-phase antigens whether the growth phase antigens have been formolized or not. The growth phase antigen, Ty O-901 was agglutinated by only five of the fifteen sera taken within the second ten-day period, and then at dilutions of 1:20, 1:40, 1:20, 1:80, and 1:40 respectively. Formolizing this culture depressed the reaction to lower levels of agglutinating titer, (1:20, 1:40, 1:20) and entirely eliminated agglutination in two of the five cases. The H antigen (0.1 per cent formolized broth culture of *E. typhi*) was not agglutinated by six of the fifteen sera taken within the second ten-day period.

The second tests in this particular series help to substantiate the impressions gained from the first. In this instance both of the growth-phase antigens were agglutinated at some titer. However, this was very low when compared to agglutination titers usually found at this time. In this, the final test in this group, all but three cases in fifteen agglutinated the H antigen at titers above those usually considered diagnostic for typhoid fever.

History of some sort of previous vaccination was obtainable in five of the fifteen cases, no one of which was more recent than five years.

AGGLUTININS IN VACCINATED INDIVIDUALS

In order to determine whether or not typhoid-paratyphoid vaccination produced O agglutinins in the inoculated individual, serum was taken from thirty medical students who were to be vaccinated against typhoid-paratyphoid prior to going to summer training camp. Thirteen of these men claimed they had never received typhoid-paratyphoid vaccine, nor had had typhoid fever. Testing their sera with the five antigens used in the previous experiments it was found that all but five of them agglutinated the

alcoholized antigens at some dilution ranging between 1:20 and 1:240, the average falling between 1:80 and 1:120. On the other hand only five agglutinated the growth phase O antigen and at dilutions of 1:20, 1:20, 1:40, 1:20, 1:40, respectively. None of them agglutinated the formolized growth phase O antigen (Ty O-901, plus 0.1 per cent formol). Of the seventeen who reported previous vaccination in some amount, all of them agglutinated the alcoholized antigens at some titer between 1:40 and 1:640. All but six failed to agglutinate the growth phase O antigen, and ten failed to agglutinate the formolized growth phase O antigen.

These results are not clearly differential. They might have some bearing on the situation if the investigation could have been stopped at this point. But when it was found at two, four, and six weeks following the routine vaccination administered by the medical officer in charge of the Reserve Officers Training Corps work at Baylor University, that the serum of all of the previously non-vaccinated men now agglutinated all of the O antigens at some titer between 1:120, and 1:640, it became apparent that O agglutinins are produced by the typhoid-paratyphoid vaccine prepared by the U. S. Army Medical School. There is little reason to hope, therefore, that typhoid can be diagnosed simply by determining the presence of O agglutinins in the sera of previously vaccinated individuals.

TYPHOID AGGLUTININS IN CHRONIC ARTHRITIS

The opinion stated above seems to be substantiated in the findings from a similar study carried out with serum from cases of chronic streptococcic (?) arthritis taken during periods of acute exacerbation. These sera were taken for another study but were available for this particular investigation of the possibility of the presence of O agglutinins in the serum of individuals acutely ill with some disease other than typhoid.

Thirty samples of such serum were studied. Thirteen of the thirty had had a full or partial typhoid-paratyphoid prophylaxis at times ranging between two and fourteen years previously.

Seventeen denied ever having had typhoid-paratyphoid vaccination.

Only two of the thirty sera from individuals previously vaccinated and not previously vaccinated, failed to agglutinate at some dilution between 1:80 and 1:1280, either or both of the alcoholized antigens. The average agglutinating titer in this series was between 1:160 and 1:240. Furthermore, none of the serum from the non-vaccinated cases agglutinated the "H" antigen, and only five of the thirteen vaccinated cases furnished sera which agglutinated the H antigen in dilutions more than 1:40.

The results in this series with the growth-phase antigens do not allow any sweeping conclusions. The serum from a small group (three) of the vaccinated cases agglutinated the formolized growth phase culture at dilutions of 1:40, 1:20, 1:20, respectively, while the serum of a somewhat larger group (seven) of the vaccinated cases agglutinated these antigens at much the same titer. None of the samples of serum from the non-vaccinated cases agglutinated the growth phase antigens, formolized or non-formolized.

DISCUSSION

From these somewhat contradictory findings it does seem to be apparent that O agglutination can and does take place under conditions other than typhoid infection. It seems, therefore, that the diagnosis of typhoid fever cannot be made simply upon the demonstration of the presence of O agglutinins in the patient's serum.

Topley and Wilson held that a case may be made for this method if a sufficiently higher titer of agglutination be set as a standard. Such a test would demand: first, the use of a rigidly standardized antigen both for qualitative and quantitative principles, and, second, a titer high enough to rule out completely the "positive" findings of non-specific origin.

The level which they proposed as diagnostic is 1:100, but from my study it seems that this would allow the test to be of value only after several weeks of the illness. Then, too, it must be recalled that many of the group of Reserve Officers Training Corps students in this study developed O agglutinins for the

growth-phase antigen to even higher titers than 1:100 following their vaccination. For this reason the possibility of the serum of a previously vaccinated individual developing a high titer of agglutinins to an infection other than typhoid must not be overlooked.

It further appears that artificially prepared "O" antigens are of no value unless especially high titers are to be taken as diagnostic and even then it would seem necessary in this instance that the comparative tests (Dreyer) be used to show that such a reaction might not be due entirely to anamnestic reactions observed at the onset of any infection.

It is neither the purpose nor the result of this study to make a case against O antigens and O antibodies. Certainly the constant finding of some amount of O agglutination during the course of an illness otherwise impossible to diagnose as typhoid is of great value, and may go a long way toward clearing up the now troublesome ambiguities of the Widal test.

AGGLUTININS IN ENDEMIC TYPHUS

With regard to the somatic and flagellar antibodies in endemic typhus fever, the material available for a study comprised only seven cases, in each of which one or two Weil-Felix tests were done using 0.1 per cent formolized suspensions of *B. proteus* X19 as antigen.

All of these cases were, clinically, endemic typhus in which the diagnosis was firmly supported by positive MacNeil (scrotal swelling) reaction in male guinea-pigs inoculated intraperitoneally with 10 cc. of fresh blood from the patient. In all of these cases positive agglutination of the formolized (H) antigen was observed as titers above 1:240, and in one instance as high as 1:2560. Identical agglutinations were obtained with 2.5 per cent alcoholized broth cultures of *B. proteus* X19. Felix⁴ stated that epidemic typhus causes the formation of O agglutinins only which are not detectable by formolized antigen. In view of our findings in endemic typhus in north Texas it might be a point of differentiation between the two diseases, Old World and New World typhus,

to observe that endemic or New World typhus seems to cause the development of both O and H agglutinins for *B. proteus* X19.

SUMMARY

1. Alcoholized *E. typhi* were used as O agglutininogen in agglutination tests carried out serially in six cases of typhoid fever. Positive agglutination was observed earlier than is usually observed in the Widal test.

2. Growth-phase selected O agglutininogen was positively agglutinated later than is usually found in the Widal reaction.

3. Formolized growth-phase O agglutininogen was agglutinated even later than untreated growth-phase selected agglutininogen and at much lower titers.

4. Both O and H agglutinins were found in the sera of typhoid-paratyphoid vaccinated individuals. The same individuals did not have O agglutinins prior to vaccination.

5. Both O and H agglutinins were found in the sera of individuals suffering with acute exacerbations in chronic arthritis.

6. Both O and H agglutinins for *B. proteus* X19 were found in the serum of seven cases of endemic typhus (north Texas).

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EXPLORING DEATH*

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It is indeed an honor and a privilege to dedicate a department founded for the purpose of obtaining knowledge and disseminating wisdom regarding the afflictions decimating man and thus to lead to a prolongation of happiness and life through such information. I might remind you briefly that man's view of death is flavored by his knowledge of the subject. Our colleague and recent contemporary, Aldred Scott Warthin, of the University of Michigan, calls life a tragicomedy in three acts: I. Evolution; II. Maturity; III. Involution, synonymous with infancy and youth, maturity and senescence each with individual characteristics. He points to the biologic span of life and its termination in normal or biologic death but this is not the only form of death that may come to the multicellular animal organism, nor is it the usual one. Unfavorable factors in the environment may check the career of the individual at any time in its course—pathologic extrinsic death, the most common fate of animal life, or inherent abnormalities may be present in the germ-plasm of any given line foreordaining its early or premature termination—pathologic intrinsic death (inherited). Very few, if any, human beings achieve a biologic span of life and a normal intrinsic death; the great majority succumb to a pathologic extrinsic death, a smaller number to a pathologic intrinsic death.

Death is of universal interest to the ecclesiast, to the poet, to the musician, to the artist, to the jurist, to the mother and father, and most to the physician. The latter assists at birth and is

* Address read at the dedication of the Department of Pathology and the opening of the new autopsy room of the School of Medicine of the Louisiana State University and the Charity Hospital Medical Center. The dedication was made on May 9, 1932 by the American Society of Clinical Pathologists, Dr. H. J. Corper, President, Dr. A. S. Giordano, Secretary-Treasurer. The laboratories of Pathology are under the direction of Dr. Rigney D'Aunoy.

continually engaged in preventing death, the two most important periods of life. Physicians and scientists through centuries of investigation laid the foundation for modern man's rational acceptance of death, bringing the conception from a gruesome tragedy to the present day idea. It is an ever changing panorama of life which has found expression variously in man's emotions. The Toten-Tanz or Danse Macabre (Dance of Death) Motive which for more than half a thousand years had an extraordinary vogue in the literature and art of Middle Europe is illustrative. Its origin is unknown; we do not know the name of the artist who first painted a Dance of Death, or that of the poet who produced the first literary form of the Motive. Probably originally it was a church play, performed within the church or churchyard, for the religious didactic instruction of the people. During the early Christian centuries the *carpe diem* philosophy of the ancients gradually became replaced by that of the Christian *memento mori*. Faith became superstition in the Dark Ages among the uneducated and ignorant people; the remnants of learning and spiritual life were found only in the church; the laity could scarcely read or write. In order to enforce its teachings of a moral life, the church found that its most efficient method was in the emphasis laid upon death, the last judgment, and the alternative future of heaven or hell. The most satisfactory explanation of the origin of the Dance of Death motive is to be found in the psychology of the times. The Middle Ages felt the primitive horror of death and expressed it in the form of the putrescent cadaver. Death was thought of only in its horrible and gruesome aspects, of the consolations of death the Middle Ages had no conception; the fear of the agony of death transcended all other emotions. To the mind of the period, the visions of the apocalypse made special appeal. What else could be expected from minds exposed to daily contact with the danger of death; in the cities during these centuries pestilence almost day by day claimed its victims by the thousands. Out of all these sources, liturgy, sermons, mystery plays, legends and poems, together with the morbid psychology and superstitions of the people there evolved a great folk cultural idea which took form in the Dance of Death as expressed in the

great wall paintings and woodcuts presenting a satire of social equality and its significance in the cultural evolution of modern society and which cannot be disregarded. The main themes of the Dance of Death are as potent today as ever they were, though altered in their significance; and they will retain their value for the human mind as long as the race persists. The physician played a potent part in all this and is revealed as he appeared to the layman, and in the latter's opinion of him. The changing social standing, professional manners and mannerisms, the progress in the knowledge and the practice of medicine and a true picture of the type of man who became a physician is revealed. Finally, in modern art we have catalogued the different forms in which death lurks for his victims. The progress made in the material world, railway trains, motor cars, airplanes, reflect new forms of death. The modern has lost his fear of death and meets it with resignation or bravery, or with cynical indifference and to this the physician and scientist has contributed. Better understanding, painless operations, bacteriology and disinfection, decrease in contagious and infectious diseases, finally a gain in knowledge of the true state of fatal human ills by more post-mortems and removal of superstitions and prejudices has brought us to the present views.

In music there have appeared many short themes of death but none can approach in picturesqueness the symphonic poem "Danse Macabre" from the pen of the talented French composer, Camille Saint-Saëns (1835-1921) inspired by the verses by Henri Cazalis (1840-1909), a poet with a penchant for gloomy and grotesque subjects. The theme is that of a dance of death from the stroke of twelve to the crow of the cock announcing the approach of day.

Inspired by the famous painting (1880) of the Swiss-German artist, Arnold Böcklin, Sergei Rachmaninoff, the Russian-American composer, wrote (1906) the remarkably descriptive and unusually beautiful tone poem "The Isle of Death" with the "Dies Irae" theme of antiquity interwoven.

Dies irae dies illa,
Solvat saeculum in favilla,
Teste David cum Sibylla.

The day of wrath, that dreadful day
Shall the whole world in ashes lay,
As David and the Sibyl say.

The latter theme (*Dies Irae*) was also introduced by Ernest Schelling, the outstanding modern American composer in "The Victory Ball" (1922) based on Alfred Noyes' poem of this name, after returning from Europe still very much under the impression of the cataclysm and finding so few remembered what the war really had meant, with its sacrifice of life and youth. A brilliant polonaise is first heard with the rhythm of the fox trot and tango mingling through it. Then there is a dramatic interruption as the vision of the dead appears before us, then two trumpet calls and the *Dies Irae* and revel again commences. Through its sensuous strains the procession of the dead continues. The bag pipes of a Scotch regiment pass and a mighty fortissimo develops. Then there is a long roll on the drums and a trumpeter sounds "Taps."

Death has held a large part of the arena even in modern art. In the Widener Memorial Library at Harvard University at Cambridge, Massachusetts, is seen a beautiful example of the conflict between death and victory, a mural painting by John Singer Sargent, the genealogical product of New England. While victory claims the hero for apotheosis death holds him for its own. How well the pathologist could look upon this theme as making life safe through exploring death, a suggestion for an artist and an analyst!

Death can be conceived as a natural and physiologic process, necessary in the scheme of existence. It can be accepted as such, and happy is the man who can do so. The Dance of Death idea is as immortal as the life of the race; but in each new period of human thought it will express itself in new form corresponding to the predominant philosophy of that time. Behind that philosophy will be seated the material knowledge and wisdom of the pathologist, the scientist, and the physician. His work will be to explore death in its dynamic aspects.

I have purposely refreshed your memory on the emotional side of death as an introduction but no narrative of death would be complete at present without introducing you again to a few of the pioneer explorers of death and to the part such explorations have played in our medical knowledge as well as to the absolute neces-

sity of every medical student and physician being groomed in this subject.

Just when postmortem examination or the exploration of dead bodies began for the sake of learning about disease, it is difficult to say but we do know that there were always small groups of students who for the sake of gaining knowledge were willing to risk punishment at the hands of then existing laws. Ancient physicians recognized many diseases but their knowledge of the human body was so scant that they were incapable of comprehending the significance of the mechanisms of many diseases beyond the fact that they produced death. Their theories were usually all wrong. Galen (130–200 A.D.), as you remember, was the first to dissect the muscles and describe them (in apes). He also wrote on the physiology and pathology of the body but in the light of modern experimental medicine his hypotheses and system of medicine have been completely overthrown.

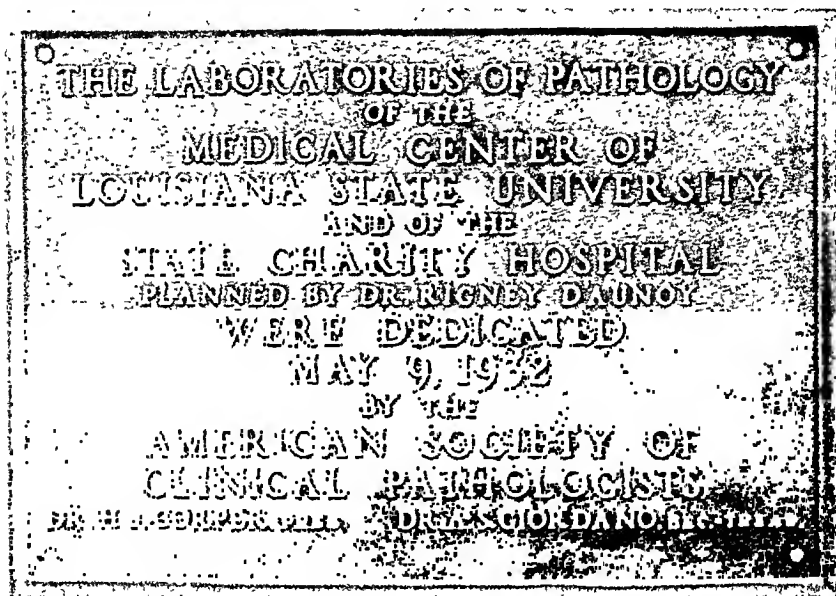
The man who first made dissecting respectable and who was the father of modern gross anatomy was Andreas Vesalius, a Belgian, who lived during the sixteenth century. By means of actual dissection of the human body he constructed for us its gross anatomy. Just as Vesalius marked an epoch establishing modern anatomy so William Harvey, the Englishman in the seventeenth century, demonstrated the circulation of the blood signaling the epoch of the beginning of modern physiology. In the same century Malpighi founded histology and descriptive embryology demonstrating the structure of the lungs, of the kidney and spleen, and of the capillaries.

The ideas held by scientists regarding the nature of disease was in a chaotic state in the seventeenth century. One school looked upon disease as an obstruction of blood vessels, another school taught that disease was explainable in terms of acidity. Still others held to the theory of vitiated humors.

Modern pathology began with the insistence of Giovanni Battista Morgagni (1682–1771) upon thoroughness and accuracy of detail in the study of morbid anatomy. Sydenham in the seventeenth century had emphasized the importance of accurately reading the story of disease from the living sick body but Mor-

gagni in the eighteenth century stressed the importance of reading with scrupulously careful, naked eyes the gruesome story from the telltale marks and lesions that disease leaves upon the bodies of those that it has slain, and attempting to correlate them with the symptoms which the patient presented during his illness.

The early nineteenth century illustrated in René Laennec the foundational value of careful and extensive studies in the death house, the postmortem room. An early intensive pathological



BRONZE TABLET PLACED IN THE DEPARTMENT OF PATHOLOGY OF THE MEDICAL CENTER OF THE LOUISIANA STATE UNIVERSITY

experience by Laennec wrought a revolutionary change of approach to the problems of diagnosis. Now a really objective examination of organs, particularly those of the chest, was made possible. By comparing stethoscopic findings on the living patient with postmortem findings, Laennec elucidated the conceptions of various diseases, and in particular tuberculosis.

At the end of the nineteenth century there appeared on the horizon a new concept, cellular pathology, introduced by the greatest pathologist the world has ever known, Rudolph Virchow

of Berlin. He maintained that the cell constituted the ultimate basis from which knowledge of both health and disease is to be derived, here the ravages and telltale marks of disease are best to be read, a lesson made possible largely by marked improvements in microscopes. The end of this century marked the death of the doctrine of spontaneous generation with the discoveries of Louis Pasteur (1822-1895) and the founding of the science of bacteriology; and parasitic diseases by prevention and treatment began to decline or disappear. The picture of death was changing.

I need hardly stress before this group the tremendous importance to the physician and mankind as a whole of exploring death and yet I cannot refrain from citing at least a few outstanding examples in modern times in which the postmortem room was invaded for material assistance and for knowledge, which you can no doubt multiply by hundreds of other examples from your own experiences.

Since this is the semicentennial year of the discovery of the tubercle bacillus and since Robert Koch is rarely referred to as a pathologist, it is appropriate that I remind you that he made frequent use of the death house for observation, study and especially for material. Those who had the pleasure of knowing Koch intimately knew that of all his studies the one concerned with tuberculosis was dearest to him and when he performed the remarkable feat of solving the etiology within a strikingly short time he resorted time and again to his pathologic experiences and to the death house for his materials. Likewise the study of Asiatic cholera in the fall of 1883 took him to the death house of the Greek Hospital in Alexandria, Egypt, where the characteristic rod was found in ten postmortem examinations and again at the Medical College Hospital at Calcutta where thirty-two postmortem examinations were performed. His therapeutic use of various tuberculin preparations again brought him intimately into the postmortem room to note the results of the use of these agents.

You will remember also that Herman Brehmer of Germany, the founder of the first successful sanatorium for tuberculosis in the world and today the largest private sanatorium in existence, received his inspiration for treating tuberculosis from the postmor-

tem table. He noted in persons dying from consumption that some of the solitary lesions in the lungs had healed and from these observations wrote a thesis on the "Curability of Consumption" and initiated his life work.

Probably the most outstanding confession of the significance of pathology and the postmortem examination is recorded by our own William Osler. Last year the members of our Society had the pleasure of visiting the little old brick building in which he performed his autopsies in Philadelphia and were able to examine his original protocols. Starting with an interest for pathology he is found in Berlin in 1873 studying under Virchow who was then fifty-two years old and a master of pathologic detail. He avidly absorbed from the lessons of a genius and early in 1876 became pathologist to the smallpox hospital in Montreal, a position created for him. Here he laid the foundation for his career as a great clinician. Dr. Abbott tells us "The fact is not so well known, that during these years, and even earlier, in his student days, he was not only a pathologist, but also, essentially, and to a remarkable extent, a museum collector."

"Viewed in the light of these records, these specimens undoubtedly present, in visible and tangible form, the first stepping stones in a great career." "His practice of medicine is literally built up out of his rich memories of these and similar cases." His serious studies as a morbid anatomist continued for thirteen years until he went to Johns Hopkins.

Some of the characteristics of this profound student of morbid anatomy are well illustrated by a story told by his cousin, Marian Osborne.

"One day we were walking down the street together. He found it difficult to walk in the accepted sense of the term; his nature seemed too buoyant to allow him to place one foot before the other, as done by the more humdrum individuals. His was the true *joie de vivre* that never left him in spite of work and sorrow and years. On this day we were dancing along St. Catherine Street hand in hand, when an old and very seedy-looking man accosted us and asked for money. Uncle Bill looked at him with his penetrating brown eyes and said with a laugh, 'You old rascal, why should I give you money to drink yourself to death?' 'Well, Sir, it lightens the road going.' 'There is only one thing of value about you, and that is your hobnailed liver.' 'I'll give it to you, Sir. I'll give it to

you.' Dr. Osler laughed and putting his hand in his pocket, drew out some silver which he gave to the old man saying, 'Now, Jehosaphat, promise me you will get some soup before you start in on the gin.' The old fellow eagerly agreed and went away with infirmity in his step. The doctor looked after him with a thoughtful expression. 'Pretty cold for that poor fellow,' he murmured and then I found we were running after the beggar. 'Here, take this. I have a father of my own', said Osler, pulling off his overcoat and putting it on the astonished old man; 'you may drink yourself to death and undoubtedly will, but I cannot let you freeze to death.' 'Tell me your name, Sir!' 'William Osler, and don't forget to leave me that liver.'

"Virtue was rewarded two weeks later. The old man before he died in the hospital made his last will and testament, leaving his hobnailed liver and his overcoat to his good friend, William Osler."

It was at the Dead House at Blockley, with a crowd of students about him, that he was most constantly to be found. The Blockley Hospital, originally the Philadelphia Alms House, is the oldest hospital in the United States. In 1870 the University of Pennsylvania moved to property adjoining the Blockley Hospital. One could leave the University Hospital by the rear and enter the Blockley enclosure by a "postmortem gate" in the old wall. Near this gate stood a little red building, a half way house to the Potter's Field. The opportunity for postmortem studies was unusually good and here in spite of every inconvenience Osler was found. It might also interest you to know that Osler's book of medicine, a pathologists' book, attracted the attention of Mr. Rockefeller and led to the creation of Rockefeller Institute and finally to the incalculable benefit to humanity which the General Education Board has rendered with Mr. Rockefeller's money. A gift inspired by a lover of morbid anatomy, and an explorer in this field.

At Hopkins Osler always first brought visitors to the Pathological Laboratory and to "Popsy" Welch. What can better express his estimation of this branch of medical sciences!

To return to the dynamic phases of death, before closing permit me to use Peyton Rous' recent theme the modern dance of death depicted in his excellent scientific volume of that name, and to again refer to Warthin's Pathology of the Aging Process. Both feel that we are quantitatively and gradually approaching the ideal

pinnacle of the maximal century age, the biologic span of life, and that the main changes wrought by medical science have been and will be concerned with the effects upon pathologic extrinsic death and pathologic intrinsic death. Accordingly old age is inevitable and escape is only possible for those who meet premature pathologic death. They are not inclined to favor the view that old age can be deferred nor that rejuvenescence may some day be possible. Be that as it may, some just as worthy minds may contest this and remind us again of what Duclaux once said "It is because science is sure of nothing that it is always advancing." Need we cite more than electrons, the Hertzian waves and cosmic rays to awake the spirit of working on to push back the boundaries of the unknown. It is well here to remember Minot's words: "We have enthroned science in the imagination, but we have crowned her with modesty, for she is at once the reality of human power and the personification of human fallibility."

Even though we agree with Rous that the underlying actions and reactions to disease within the body remain essentially what they were in the past the pathologist will have to be on the alert to discover the early and so-called new manifestations of previously undescribed disease conditions as well as to discern the quantitative or relative significance of diseases in certain communities and at certain times. Painstaking and careful observations are as essential today as they were in Vesalius' time, or as they were for Osler.

In Utopia the necessity for the pathologist and for exploring death may disappear and I dedicate this Department of Pathology of the Medical Center of Louisiana State University and of the State Charity Hospital to a useful and valuable service for preserving the health and life of mankind in this community and in setting a good example for others to follow.

LYMPHOMATOUS COMPRESSION OF THE SPINAL CORD*

WITH A CASE REPORT OF THE HODGKIN'S TYPE

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If syphilis be accepted as the most protean of infectious diseases, certainly, that group of neoplasms falling into the general classification of lymphoma, should take first rank in its protean aspects among the neoplastic diseases.

Unfortunately, there is no generally accepted terminology for the conditions under consideration. Etiology is, to some extent, a matter of opinion, and pathogenesis is obscure. Indeed, confusion may arise in the interpretation of histologic changes observed in the same specimen of lymphatic tissue. Nevertheless, at least two factors are common to the group; first, a progressive enlargement of fixed or disseminated lymphatic tissue; and second, a fatal termination.

Lymphomatous nodes have certain characteristics. Growing expansively, the early nodules are firm, discrete, freely movable, and not tender, so long as there is no accompanying periadenitis. In older nodes, the value of these factors for differential diagnosis is relatively more limited, so that biopsy and microscopic examination are necessary to establish their identity. Frequently their gross appearances will distinguish lymphomatous nodes from those of acute and chronic infectious origin, and to some extent, the cut surfaces of the various types of lymphomas are suggestive, although seldom diagnostic.

As a rule, however, these various observations are of relative importance only and resort must be had to microscopic study.

* Read before the Eleventh Annual Convention of the American Society of Clinical Pathologists, New Orleans, Louisiana, May 6-9, 1932.

Since the histology of the lymphomatous node is relatively limited, usually it is not difficult to agree on the presence or absence of cytologic elements. It is their interpretation, classification, and terminology, which become diagnostic stumbling blocks. As a rule, the less complicated the terminology, the less confusion will arise; therefore, the simplest classification sufficiently comprehensive has the merit of being the most useful for practical purposes.

Owing to its simplicity and adaptable character, the writer has adopted, with slight modification, the classification followed by Baldrige and Awe.² While it is not my object to discuss or defend the terminology of any classification, it is desirable, because of the existing confusion, to avoid the risk of being misunderstood.

The grouping referred to, in an analysis of 150 lymphomas, is based chiefly on the cytologic criteria; and, with the more common synonyms in brackets, is outlined as follows:

Lymphoma (Lymphoblastoma) (Malignant lymphoma)

- (1) Sclerosing type (Hodgkin's disease) (Lymphogranuloma)
Fibrosis, mononuclear and multinuclear giant-cells (Dorothy Reed), lymphocytes, eosinophiles in a connective tissue reticulum, with or without necrosis.
- (2) Endothelial type (Lympho-epithelioma)
Uncommon; epithelial (or endothelial) groups of cells with poorly staining cytoplasm resembling a syncytium; may be mistaken for metastatic carcinoma.
- (3) Lymphoblastic type (Lymphosarcoma)
Cells resembling the maternal cells of a normal germinal center are found throughout the node; moderate amount of stroma; tendency to invade outside the capsule.
- (4) Lymphocytic type
 - (a) With leukemia (Lymphatic leukemia)
 - (b) Without leukemia (Aleukemic leukemia)
 Uniform small lymphocytes without stroma; a few trabeculae; looks like a sac (capsule) full of cells.

Comparatively, the histologic variations in these types of lymphoma are no greater than the architectural variations in carcinoma of the breast.

All reference to the sites of predilection of lymphomatous neoplasia has been studiously avoided up to this point, lest the importance of this factor be somewhat overshadowed. The widely disseminated and protean character of lymphomatous disease, especially the Hodgkin's type, has not been accorded due emphasis in medical texts and monographs. Ginsburg,⁹ in commenting on this anomaly, cited the example in Elsberg's recent monograph on "Tumors of the Spinal Cord." In this excellent work there is no reference to a Hodgkin's involvement of the cord; yet, in one New York hospital (Montefiore) alone Ginsburg stated that, of thirty-six patients with Hodgkin's disease from 1914 to 1925, ten were observed in which "definite invasion of the nervous system was a striking clinical phenomenon." Davidson and Michaels⁵ made a similar allusion in reporting twenty-six cases of lymphosarcoma from the same institution between 1922 and 1929, four of which showed signs of spinal cord compression. In spite of numerous isolated communications attesting to the varied manifestations of lymphomas, it is a fact that these data have not been adequately assimilated into our standard literature. There is still a widespread belief that lymphomata are mere "affections of the absorbent lymph nodes and spleen."

Although it is unquestionable that in a large series of cases, the peripheral lymph nodes are the first to attract clinical attention, there are impressive and diverse groups of outstanding presenting symptoms of disease due to lymphoid neoplasia, with but slight, if any, involvement of the peripheral glands. Leukemic infiltrations in lymphatic leukemia have, as a group, been anticipated, yet there are instances where the typical leukemic blood picture has been delayed (aleukemic states) until long after the onset symptoms of dissemination. Failure to associate such symptoms and a typical blood picture is quite inexcusable. In the sclerosing, endothelial, and lymphoblastic types of lymphoma no trustworthy aid is offered by the blood picture. The key to diagnosis will rest with the finding of a lymph node for sectioning, if perchance one be available.

That the involvement of various organs and tissues, other than

the lymphatic system, is not limited merely to a few isolated foci of academic interest and inconsequential nature, has been stressed by many authors. The crux of the matter is, that the importance of metastases and disseminations (in lymphomas) frequently transcends that of the primary pathology. The resulting clinical symptoms and signs are so diverse and manifold that, were the condition more common, it would be a problem of the first magnitude in differential diagnosis.

By way of illustration, a few of the chief and outstanding symptoms for which patients have sought relief have been: generalized and localized pruritus, paroxysmal tachycardia, melena, diarrhea, jaundice, hematuria, motor paralysis, sensory disturbances, and intermittent fever. From the diversified list compiled of other conditions simulated, one finds: Malta fever, hypernephroma, bronchial asthma, pulmonary tuberculosis, osteomyelitis, and gastric carcinoma. Of the organs and systems chiefly affected, the skin, nose, tonsils, trachea, and bronchi, stomach, intestine, liver, spleen, pancreas, osseous, nervous, and hematopoietic systems. Indeed, there is evidence to show that in the sclerosing type alone, no organ or tissue has escaped invasion.²

In a general way, the type and mechanism of dissemination may be summed up as follows: In the sclerosing and endothelial types of lymphoma, the disseminated lesions are of the nature of discrete nodules for which the term metastasis is perhaps more appropriate. In the lymphoblastic type, the lesions are not only discrete, but there is likely to be a localized destruction of the parenchyma, or an infiltration in which the lymphoid cells tend to be arranged in rows between the parenchymatous elements or surrounding the vessels. In the lymphatic type, both with and without leukemia, there are perivascular and intercellular infiltrations of lymphoid cells, but rarely any nodule formation. In long standing cases, the leukemic infiltration becomes almost universal. In general, it may be said, the younger the patient the more virulent the disease; in childhood, rapidly fatal, whereas in adults it is essentially chronic.

The invasion of the nervous system was first reported by Murchison¹⁴ in 1870, and has since been noted by Goodhart,¹⁰ Gowers,¹¹

Longcope,¹³ Askonazy,¹ Dietrich,⁶ Welch,²¹ Sternberg,¹⁸ Weber,²⁰ Pancoast,¹⁵ and others. That clinical symptoms resulting from such invasion may dominate the clinical picture is attested to by the case reports of Askanazy, Hecher and Tischer¹² Walthard¹⁹ and Reese and Middleton.¹⁷

In 1917, Basso³ collected and published the reports of cases of paraplegia proved at necropsy to have been caused by leukemic infiltration or chloroma in the spinal cord. He believed that a number of these cases have been described under that disease of the hematopoietic system known as chloroma, a condition admittedly having all the features of leukemic infiltrations with the unexplained characteristic of a grass-green color of the tissue which soon changes when exposed to the air. Green tumors are said to have been found in all forms of acute and chronic leukemia of both myelogenous and lymphatic types. Von Recklinghausen, in 1885, called attention to the similarity of the histologic picture of leukemia and chloroma, and in 1893, George Dock⁷ in analyzing seventeen cases then known, including one of his own, was fully aware of the leukemic nature of chloroma.

Dock and Warthin,⁸ in 1904, reviewed twenty-two cases, in nine of which there was involvement of the vertebrae "usually in the periosteum of the bodies, sometimes in the dura, sometimes in the fat of the vertebral canal" with atrophy and degeneration of the cord.

Among the authors of the more widely used text-books are Ewing, Aschoff, McCallum, and Negeli who divided chloroma into lymphoid and myeloid types. Mallory alone considered it a part of myelogenous leukemia. Using the oxydase reaction in blood smears as a criterion for classification, Brannan¹⁶ was of the opinion that chloroma should be considered a subvariety of myelogenous leukemia only.

From a review of the literature, Reese and Middleton concluded that the most common form of paraplegia in leukemia is due to epidural or peridural infiltration with resultant mechanical compression of the cord. Intramedullary areas of softening due to leukemic infiltrations occluding spinal cord vessels have rarely been reported.⁴

In the sclerosing type of lymphoma, peridural infiltration resulting in a collar-like mass partially encircling the cord, with compression as a sequel, seems to be much more common than intramedullary invasion. It is of interest to note also the frequency with which that portion of the spinal canal between the third and eighth dorsal vertebrae has been involved, altho true to form, lymphomatous infiltrations in the central nervous system seem to scorn any particular site of predilection: no portion is immune. Curiously, in the case of myelogenous infiltration of the spinal canal reported by Basal in 1917, the "region of the 4th, 5th, and 6th thoracic vertebrae" were found involved.

The lymphocytic and sclerosing types of lymphoma, while relatively more frequent than the endothelial and lymphoblastic types, seem to be the chief invaders of the nervous system. Baldridge and Awe reported but one involvement by the combined endothelial and lymphoblastic type in their analysis of 150 consecutive cases as against nine for the sclerosing type, and five for the lymphocytic type. Davison and Michaels reported four cases of lymphosarcoma with signs of cord compression.

As to the clinical recognition of lymphomatous compression of the cord, perhaps the most comprehensive deduction is to include this possibility in the differential diagnosis in appropriate conditions. Cord symptoms associated with a leukemic blood picture form a combination almost equivalent to diagnosis; however a temporary aleukemic state must be kept in mind. Cord symptoms in a patient with lymphomatous nodes, especially of the sclerosing (Hodgkin's) type may be strongly suspected to be due to a lymphomatous peridural color. In case neither a leukemic blood picture exists nor a lymph node is available for section, reliance must be placed chiefly on clinical symptoms alone.

Reese and Middleton felt that the syndrome of paraplegia occurring in children and young adults, with pain in the back, radiating intermittently into the legs, followed by a spastic paretic gait, later by bladder retention and rectal incontinence and finally by a complete compression syndrome associated with trophic disturbances, serves to differentiate leukemic compression from that due to other tumors. This sequence of events was remarkably closely followed in the case I wish to record.

CASE REPORT

R. K., a tenth-grade school boy, aged fifteen years, was the son of a colleague. With his father, I first examined this patient February 23, 1931. The chief symptoms at that time were nearly complete motor paralysis and numbness in both legs. The present illness dated from December, 1930, when the patient complained that his feet and legs felt heavy. On one occasion he fell down several steps without injury. His father observed that the boy's muscular movements seemed clumsy at times; also, that he frequently shifted his posture from erect to stooping and from side to side. He complained of pain in the back between the scapulae.

Following a common cold January 26, 1931, which lasted two days, the patient gradually lost weight and color, and developed an obstinate constipation, necessitating manual relief. February 21, 1931, motor weakness in both legs developed rapidly, and February 23, he last walked alone.

The patient was referred to Dr. C. Van Epps at Iowa City, to whom I am indebted for a record of the balance of the history and clinical examination. By February 26, motor paralysis in the legs had become complete, and numbness extended to the costal margins. An increasing slowness in urination culminated in the necessity for frequent catheterization after March 1st.

Examination by Dr. Van Epps, March 2, 1931 revealed: weight 141 pounds, a loss of 13 pounds in one month; height 70 inches; marked motor and sensory loss up to the midepigastrium; paralysis of the spastic type involving both legs, with increased deep tendon reflexes, bilateral ankle clonus, and bilateral plantar extension. Clinical impression: meningo-myelitis with compression at seventh thoracic segment.

Laboratory data; Blood: erythrocytes, 4,850,000; leukocytes, 8,900 to 11,200; hemoglobin 90 per cent (Dare); neutrophils, 73 per cent; eosinophils, 2 per cent, lymphocytes, 20 per cent; unclassified, 5 per cent. Wassermann negative. Cerebrospinal fluid: Pressure, 2 mm. Hg. increased slowly to 6 mm. Hg. on coughing; fluid, clear; 3 lymphocytes per cu. mm.; globulin, 151.9 mgm. per cent; sugar, 79.0 mgm. per cent. Cerebrospinal fluid, March 5: Pressure, 8 mm. Hg. increased slowly to 14 mm. Hg. on coughing; eight lymphocytes per cu. mm.; globulin, 183.0 mgm. per cent; sugar, 78.0 mgm. per cent. X-ray of spine: anterior-posterior and lateral views: Negative.

March 19, 1931, a laminectomy by Dr. H. Bye extending from the third to the eighth thoracic vertebrae, revealed a thickened compact area of peridural fat at the fifth vertebrae, also an extradural collar-like mass, approximately 10 cm. wide, compressing the cord. Microscopic examination of tissue from the spinal canal by Dr. Hansmann, showed "small round cells with oval and round deeply staining nuclei, and a narrow rim of bluish pink cytoplasm; also large epithelial cells with paler staining nuclei; a small number of Reed giant cells, and a relatively large number of eosinophiles; and a well marked diffuse fibroblastic reaction."

The patient's recovery from the operation was uneventful, but without symptomatic relief. He suffered intensely painful spasmodic contractions of both lower limbs, resulting finally in flexed contractures and marked atrophy. He became very anemic, and emaciated, expiring January 7, 1932 from complete exhaustion, and a terminal infection.

AUTOPSY REPORT

Male body, greatly emaciated. The thighs are flexed on the body, and the legs on the thighs at angles of about 45° , so that as the body lies on the side, it forms the letter Z. The joints are not ankylosed; both legs and thighs can be extended with sufficiently great pressure as to overcome the contractures. Articulations of the upper extremities are normal; there is no rigor.

The skin is white, of fine texture, and the mucosae are very pale. There is a moderate oedema of the feet, ankles, and in the lumbar region. The hair of the head is black and dry, the body hair is scanty. The external genitalia and rectum are normal. The external auditory and nasal passages are negative. The eyelids are approximated, the sclerae are pale, the pupils are dilated, equal and regular. There are no palpable lymph nodes. There are no external anomalies. Aside from the adduction and acute flexion of the thighs and legs, there are no deformities. There is a 25 cm. healed surgical incision extending down the mid-line of the back, beginning 5 cm. below the spine of the seventh cervical vertebra.

On median section, the subcutaneous tissues are oedematous; the panniculus is very thin and orange colored. The blood is thin and dark. The abdomen contains no free gas or liquid. The urinary bladder is distended with ammoniacal urine; the walls are thin and parchment-like. Other abdominal pelvic viscera are of normal size and position.

The costal cartilages cut easily. The marrow of the sternum is light red and granular. There is approximately 1300 c.c. of blood stained fluid in each thorax. The mediastinum contains large irregular masses of lymphatic nodes arranged roughly in three groups.

(1) In the region of the thymus remnants, there are from twenty to thirty nodes, some discrete, some coalesced and matted together, varying from pea to hickory-nut size. These nodes and the interglandular connective tissue are very adherent to the larger vessels, so that it is almost impossible to dissect the mass free without cutting into vessel walls.

(2) A second group is composed of one large rhomboidal mass occupying the space between the sternum and vertebrae, adherent to the right and posterior aspects of the trachea. Grossly, this mass consists of one large node anteriorly and a fringe, and small coalesced nodes posteriorly.

(3) A third group of nodes is in the bifurcation of the trachea, and extends both right and left surrounding the bronchi. There are approximately fifteen of these nodes, some of which are coalesced, others discrete, and all very adherent to the trachea, bronchi, and perivertebral tissue posteriorly. On section grossly,

the smaller nodes in each group present a smooth, pink, shiny surface. The medium sized and larger nodes show more or less patchy fibrosis. The largest nodes in the bronchi region show extensive fibrosis with streaks and trabecula of lymphoid tissue. This group of nodes is also adherent to the ventral surface of the perivertebral fascia, particularly over the body of the fifth thoracic vertebra, but also over-lapping the fourth and sixth vertebrae. Removal of the glandular mass from the vertebral bodies reveals marked changes in the bodies of the fourth, fifth, and sixth vertebrae. The body of the fifth is much softer, and more granular. The bodies of the fourth and sixth are distinctly softer and more granular than normal, also less so than the body of the fifth. These bones may be cut into with a knife, and on section show numerous pinhead to pea-sized masses of a light pale green, jelly-like substance. Within the spinal canal and around the dura there is a band-like mass of firm light green colored material nearly completely encircling the dura. It is as though a ring had been cut and spread apart slightly. The spinal cord is distinctly compressed to about one-half size within the dura directly beneath this band. The band varies from 4 to 6 cm. in width. On each side of the band, both up and down the dura for a distance of 5 or 6 cm., there are pea-sized masses of this same soft light green material. Peridural compression of the spinal cord is very clearly demonstrated.

The pericardium contains about 25 c.c. of clear straw colored fluid. The heart is a little larger than the cadaver's fist. The muscle is pale. The valve flaps and orifices are of normal size. There are no vegetations nor deformities. The endocardium is smooth and pale.

The lungs are air containing throughout. The hylum of each lung shows several large peribronchial lymph nodes, having the same appearance of those in the mediastinum. The larger nodes contain much more connective tissue than the smaller.

The liver is of normal size and consistency; the cut surface has a fatty sheen. The bile passages are free of exudate and concretions. Serial sections show no gross changes, other than the obvious fatty change. A careful search for nodules revealed none.

The spleen is of normal size, a little softer than normal; on section dark red and moist. There are no gross changes, no nodules.

Both kidneys show multiple pinhead sized masses on the surface, yellowish white in color. The pelves of the kidneys and ureters are patent, not dilated, and have a moist shiny surface.

The adrenals are of normal size, and show no gross changes. The retro-peritoneal hemolymph nodes appear normal in size and on section.

The gastro-intestinal tract shows no gross change aside from the pale color, which is everywhere apparent throughout the peritoneum.

The urinary bladder is distended and contains about 1000 c.c. of amoniacal turbid urine. The wall is parchment-like. Both testes are in the scrotum, and appear normal on section.

The bone marrow from a rib is light red, cellular, and less moist than normal.

MICROSCOPIC REPORT

Mediastinal lymph nodes: The smaller nodes reveal a fine fibrillar reticulum forming a ground substance for lymphocytes, eosinophiles, mononuclear and multinuclear giant-cells. The larger nodes are less cellular, the connective tissue reticulum is much more dense and abundant, and the normal architecture of the node is rearranged—completely lacking in the larger nodes.

Liver: The cells are pale staining, and have a ground glass appearance. The cords are somewhat broken up. The walls of the veins are thickened. The sinusoidal spaces contain a few polynuclear cells and larger cells containing a brown pigment (mononuclears).

Spleen: Histologically, the spleen is well preserved. The splenic arteries contain groups of cells of the lymphocytic type, while the veins are distended with red cells. There is a moderate cellular hyperplasia, and an occasional field showing eosinophiles.

Bone Marrow: There is a very fine, delicate reticulum. The cells constituting the mass of the marrow are vesicular, with pale-staining nuclei, and a small rim of protoplasm. There are less than the normal number of erythrocytes, none of which are identified as containing nuclei. The capillary and sinusoidal network form irregular cords and groups.

Sections from the fifth thoracic vertebra show an enlargement of the marrow spaces and a rarefaction of the bony trabeculae. The spaces are filled with cells resembling the large lymphocytes. There are a few cells having eosinophilic granules. There is a delicate fibrillar network more marked in some fields than in others. It is apparent that cords and groups of these cells resembling large lymphocytes extend from the marrow spaces of the vertebral body through the periosteum, through the prevertebral fat and fascia, and into the spinal canal. By direct extension through the lymphatics, blood vessels, and between the bony trabeculae there is a continuity of the bone marrow new-growth and the collar-like peridural mass. Histologically the structure is the same.

SUMMARY

(1) Attention is called to the protean character of the manifestations of lymphomatous neoplasia.

(2) A case of lymphomatous compression of the spinal cord is reported with clinical, laboratory, and histologic data.

(3) The principal pathologic changes noted are:

(a) Three groups of greatly enlarged, coalesced lymph nodes, very adherent to surrounding structures in the mediastinum, around and behind the bronchi.

(b) The adhesion of the lymph nodes to the prevertebral fascia.

- (c) The marked softening and granular appearance of the bodies of the fourth, fifth and sixth vertebrae, and the presence therein of a light pale green gelatinous substance.
- (d) Compression of the spinal cord by a collar-like mass most marked at the level of the 5th thoracic vertebrae.
- (e) Continuity of cellular changes within the vertebral bodies, and the peridural collar-like mass.

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"SENSITIVITY" TO SULPHYDRYL

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In a report⁶ on the use of compounds of the sulphydryl group, in particular, thiocresol in wound healing, it was stated that after a short time of application a certain number of individuals, so treated, developed an itching rash. It was found during the course of application of sulphydryl compounds (thiocresol, benzyl mercaptan) to the skins of rats and mice that a certain percentage of these animals also reacted in a similar way.

In order to obtain an estimate of the number of humans who reacted to this group, 450 "normal" individuals, 290 women and 160 men, aged from eighteen to seventy years, were subjected to the following procedure. In addition, for reasons given below, thirty-two women and forty-five men with known carcinoma were also tested.

The skin in the cubital space of the elbow was chosen for convenience. Thiocresol in 1 per cent alcohol solution was painted once on the right arm; the same concentration of cresol in the same solvent was used on the left arm. Observations were made for a period of three weeks.

RESULTS

Eighteen of the "normal" individuals, ten women and eight men, were found to react, (see fig. 1). Among the individuals with carcinoma, five reacted. The time of appearance of the reaction varied from three to eighteen days. None reacted to cresol.

In those who did not react, no sensation whatever was noted and no objective findings could be discovered. The first symptom in those who did react was an itching, which grew in intensity until it equaled that of well developed ivy poisoning, (to use the comparison of one of the individuals). A diffuse redness was

sharply limited to the area which had been painted, small papules and blisters appeared and itched intensely. The blisters perhaps coalesced and, in extreme cases, produced a very large bleb. The erythema disappeared from between the papules and vesicles, allowing them to become more prominent, they then gradually dried, a few crusts were formed, and finally the whole area showed mild desquamation. Red to brown pigmentation remained for several weeks more.

DISCUSSION

The facts that certain chemical groups in complex molecules elicit response in different individuals, and that rearrangement

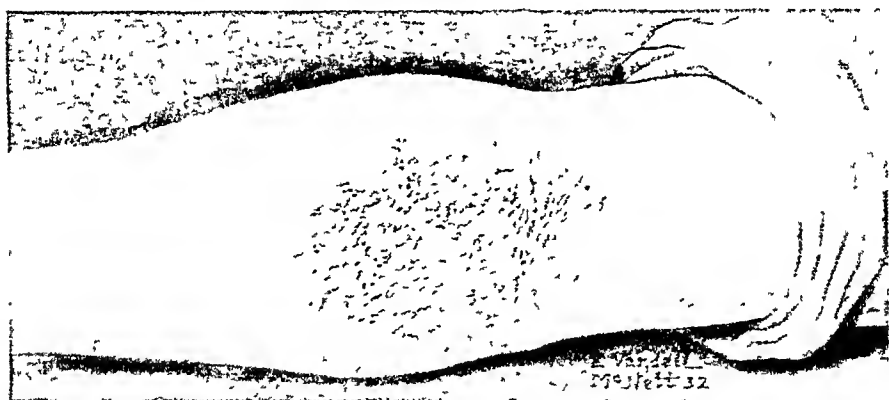


FIG. 1. REACTION ON ARM OF NURSE TWO WEEKS AFTER APPLICATION
Nothing appeared on the control arm

of these groups in the molecules changes the conditions. have been brought out especially in the studies of Landsteiner and associates.³ That this is an example of such a phenomenon seems quite probable.

Since the thesis that the sulphydryl group is essential to cell division is now established,¹ the first thought is that those individuals who are sensitive to this group are also sensitive to cell division phenomena. It will require time and much more data to discover this relation, if present.

The eruption on the skins of the rats and mice which exhibited

this reaction disappeared in about two weeks, in spite of continued painting with the material. The same end result was found at the end of about six weeks as in the animals not "sensitive," namely, thickened skins, with no histological differences.^{2,4,7}

Five patients with local ulcerating surfaces were treated with the 1:10,000 watery suspension of thiocresol to promote healing.⁵ The original carcinomas had been treated by wide surgical excision in three and by cautery in two. In all five the eruption appeared around the edges of the wounds after about a week and treatment was discontinued. These also were the patients with carcinoma who reacted positively as noted. We have not treated any other patients with wounds left from removal of carcinomas.

An observation recorded in two young women is to the effect that after intervals of three and two weeks respectively, after painting, with no sign of reaction, the eruption appeared suddenly on the first day of menstruation.

SUMMARY

Local areas of the skin of the arms of 450 "normal" individuals were painted once with 1 per cent alcoholic solution of thiocresol and controlled on the other arm with one per cent cresol solution. Of these, eighteen individuals reacted by the production of an itching rash.

Among seventy-seven individuals with carcinomas similarly treated, 5 reacted, all of whom had open, ulcerating wounds; the region around the wound also reacted.

The special significance of this "sensitivity" to the sulphydryl group is unknown, but it is probably related to the general phenomena of sensitivity to specific chemical groups and arrangements. Since the use of the sulphydryl group in p-thiocresol and other compounds is increasing, attention is drawn to this phenomenon. At present it is recommended that in those who react, treatment be discontinued.* It is also recommended

* Bogert and Husted (*Jour. Pharm. & Exp. Therap.*, 45: 189-207. 1932), call attention again to the susceptibility of certain individuals to the benzothiazoles in which similar latent periods are described as were encountered with -SH compounds.

that such treatment of ulcerating carcinomatous wounds be avoided in this group.

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EDITORIAL

LABORATORY SERVICE FOR HOSPITAL RESIDENTS

Not the least vexing of the many problems confronting the average hospital is the arrangement of the Resident's service in the clinical laboratory so that the experience may be of practical value in his later career.

This difficulty arises in part from the fact that the allotted period is usually too short to permit either thorough training or a comprehensive survey of this phase of the practice of medicine; and in part from the frequent tendency of Residents to regard this phase of their hospital career as a period of true servitude, indeed. Lacking in the drama of the accident service, and the operating room, impersonal in the material with which its work is concerned, the laboratory service is often of importance to the Resident only as standing between him and these promised lands; something to be encountered with resignation and encompassed with relief.

The laboratory is not always absolved of responsibility for the perpetuation, if not the development, of this attitude. Lacking the skill and experience for the more specialized laboratory procedures, too often the Resident spends his time in the examination of urine and the counting of blood cells, procedures important enough in themselves but likely to become monotonous when confronted in bulk. And it may be questioned with justice if the return to the Resident is commensurate with the time expended and if such a plan best achieves the purpose in view. It cannot be too strongly emphasized that the purpose of such a laboratory service is not the acquisition of technical skill.

While it is impossible in these modern days to escape utilization of the resources of the laboratory, it is also inevitable that these are best applied through the medium of those skilled in their use.

In the last analysis the profitable utilization of the laboratory in the study of disease depends not so much upon an extensive

knowledge of technic but upon a thorough appreciation of what to do, when it may be most profitably done, and above all, what is its clinical significance and utility when it has been done.

In other words, it is not the test but its clinical significance and application which is of paramount importance, and emphasis of this fact should be the primary purpose of the Resident's laboratory service.

Technical facility cannot, however, be entirely neglected; some things the Resident must be taught; first, those which he may be called upon to perform as emergencies in the absence of the regular laboratory force and, second, those which, in the early days of his professional career, he may well utilize in the routine examination of the patient.

It is of great practical value, also, that in the collection of specimens for varied purposes, he acquire a thorough and relatively extensive training in venipuncture, both because the necessity will inevitably, and even frequently, arise in the course of clinical practice, and because lack of skill in this simple procedure transforms it into a gory performance regarded with apprehension by the patient, attended by unpleasant and, perhaps, even serious sequelae, reflected in an unfavorable, even though unjustified, estimate of the ability of the physician.

Every endeavor, therefore, should be made to present the laboratory service to the Resident in this light. He should learn by observation the proper methods for the collection and forwarding of laboratory specimens, for the success or failure of laboratory examinations, the reliability of their results, and consequently their significance may often be directly dependent upon the proper collection and after treatment of the specimen.

He should learn, by observation, reading, and discussion how to select from the multiplicity of available procedures those most likely to be informative in a given case and, particularly, how to interpret and utilize their results.

Whatever he does, or sees done, he should be primarily and acutely interested in its clinical significance, should endeavor to form his own estimate and should check this by observation of the subsequent course of events and by discussion or reading.

He should endeavor to make it a habit when collecting or examining a specimen, to ask himself:

1. What information will this examination furnish?
2. Would any other procedure be equally, or even more, useful?
3. What other information can the laboratory furnish in the study of this case?
4. Is this procedure related to: a) diagnosis; b) prognosis; or c) is it a means of suggesting appropriate methods of treatment or measuring their results?

The end and aim of the entire service, in short, should be to emphasize the fact that laboratory procedures are not merely "tests" but methods designed to ascertain and measure the response of the body to varied stimuli and to interpret these phenomena in terms of reaction to disease.

—R. A. KILDUFFE

NEWS AND NOTICES

THE TWELFTH ANNUAL CONVENTION

The Twelfth Annual Convention of the American Society of Clinical Pathologists will be held in Milwaukee June 9-12. A very elaborate program has been planned, however the local and program committee is not able at this time to give all of the details. An outstanding event will be a Symposium on the Medico-legal Autopsy which promises to contain one of the most useful groups of papers presented at our meetings.

The Program Committee would like to have authors send brief abstracts (not more than 200 words) of their papers at the earliest possible moment. These will be published in the May issue of the JOURNAL. This is purely voluntary on the part of the author and is being done as an experiment to see if it is worth while. Abstracts cannot be published unless they are in by April 10th.

The Scientific Committee would like to urge members to bring demonstrations of various tests, material and apparatus and to notify the Secretary as soon as possible concerning the amount of space desired.

The Research Committee would like to receive as much material as possible between now and the meeting in order that an adequate report can be made of it.

Clinical Pathologists and laboratory technicians will be interested in the following announcement:

Beginning April 1, 1933, applicants to the Registry must pass an examination conducted by a member of the American Society of Clinical Pathologists practicing in the locality in which the applicant resides. This examination will comprise:

- (a) An oral and practical test, counting fifty per cent,
- (b) Written test, twenty-five per cent,
- (c) Personal and psychological attributes, twenty-five per cent.

The fee for registration is Ten Dollars and is not returnable in case of failure. The applicant may, after the lapse of six months, be given the privilege of another examination without additional charge.

A notice has been received to the effect that the Journal of Preventive Medicine has been obliged, for financial reasons, to suspend publication at the end of the sixth volume. The American Journal of Hygiene will annually publish a number devoted to papers of the type which normally appeared in the Journal of Preventive Medicine. This number may be subscribed to separately.

The attention of clinical pathologists is invited once more to Biological Abstracts. The editors have succeeded in bringing out the Index to the second volume which is strongly recommended to members of the Society. This Index is the most comprehensive thing ever undertaken along this line for it is not only an index to abstracts but is a correlated summary of biologic sciences and shows clearly the inter-relation of different phases of biology. To the clinical pathologist this is a most valuable contribution and all members of the Society will profit by studying this Index volume very carefully. Other indices are in press and will be forthcoming shortly. Biological Abstracts needs support more than it ever did before. If this project of abstracting biological literature fails even the most optimistic doubt that it will ever be attempted again for years to come. The vast number of publications in the field, especially those dealing with medical subjects, is getting to be so large that the necessity of these abstracts becomes all too apparent to even the casual reader. Subscribe to Biological Abstracts and lend your support to this most worthy movement!

The LaMotte Chemical Products Company, regular advertisers in the JOURNAL, have specialized and originated the practical application of hydrogen ion concentration control. They have developed indicators, buffer salts and buffer mixtures as well as apparatus and sets for determining the hydrogen ion concentration of bacteriological culture media, sera, milk, urine and other fluids. Clinical pathologists can be assured of obtaining reliable reagents from this Company.

BOOK REVIEWS

Antony van Leeuwenhoek and His "Little Animals." BY CLIFFORD DOBELL. Pp. 435. London, New York, Harcourt, Brace and Company, \$7.50.

Exactly 300 years ago there was born a simple unlettered Dutchman, who, at the age of forty-one, startled the Royal Society of London by announcing that he had discovered a new world. It was a world of little animals—"animalcules"—some so small that a thousand million of them were no larger than a grain of sand. He so convinced the ultrascientific Fellows of The Royal Society as to the truth and novelty of his observations that they made him one of their number. And in those days, as now, this was no small honor, because The Royal Society of that early date contained in its membership such intellectual giants as Robert Boyle, Robert Hooke, Christopher Wren and the omnipresent Samuel Pepys.

Clifford Dobell celebrates the tercentenary of the birth of this strange and simple Dutchman, Antony van Leeuwenhoek, by reintroducing him to us as the "Father of Bacteriology and Protozoology." Even though most Americans have avoided knowing much about Mr. van Leeuwenhoek, largely because they have been awed by the spelling of his name, and have despaired of ever correctly pronouncing it, Dobell, with great justice, urges us to meet his remarkable friend, shake his hand, and hearken to what he has to say to us, "for he is a man worth knowing more intimately. And though he was born exactly 300 years ago he is still very much alive and would be glad to make your better acquaintance—provided that 'you are a true lover of learning' (as of course you are)."

Of course, several hundred thousand Americans have already met Mr. van Leeuwenhoek, "The First of the Microbe Hunters," in Paul de Kruif's magnificent "Microbe Hunters." But the whole story of this splendid fellow could hardly be told in one

short chapter. Those whose appetites were whetted by de Kruif's story will find rich satisfaction in Dobell's biography, a labor of love which required twenty-five years of ardent research.

After one has jumped the hurdle of correctly pronouncing his name, which is much simpler than it looks (Lay'-wen-hook), one can settle back to several evenings of delightful entertainment with Dobell's book in his lap.

Because this great and simple man knew no language other than Nether-Dutch, and because he never wrote a book or a scientific paper—only letters and still more letters—his writings have suffered severe mutilations and perversions at the hands of his translators. After Dobell had discovered that Leeuwenhoek's original Dutch letters were, for the most part, extant in the Archives of The Royal Society in London, he abandoned all attempts to learn about the man from the writings of others.

To Dobell's delight he soon found that Leeuwenhoek knew no "science," for he was merely the proprietor of a dry-goods store and haberdashery, who also held the civic appointments of Wine Gauger and Chamberlain to the Sheriffs of his native town of Delft, which means, according to the terms of appointment that it was Leeuwenhoek's duty "to clean the aforesaid Chamber properly and to keep it neat and tidy." From his small income, this draper-haberdasher-wine gauger-janitor derived sufficient funds to permit him to conduct his explorations into a new and unsuspected world.

When not engaged in his janitorial duties, or selling buttons and ribbons, or testing wines, Leeuwenhoek ground and polished lenses and built his own "microscopes"—hundreds of them. They were simple affairs, consisting of a tiny matchhead size biconvex magnifying glass, mounted between silver, gold or brass plates. With these highly original instruments he discovered the weird and fantastic world of "little animals" in well-water, seawater, snow-water, pepper-water, clove-water, nutmeg-water, or in the intestinal contents of horse flies, fleas, lice, maggots, snails, spiders, beetles, not to mention cows, calves, sheep, rabbits, whales, and human beings. He not only described with remarkable simplicity and accuracy free-living and parasitic protozoa

and bacteria, but he expressed a strong suspicion of the part played by putrefactive organisms in the general economy of nature—almost 200 years before Pasteur and Koch proved that these “little animals” were the chief murderers of mankind.

Doctor Thomas Molyneux, a contemporary Fellow of The Royal Society, after visiting Mr. van Leeuwenhoek described him as “A very civil compleasant man, & doubtless of great natural Abilities; but contrary to my Expectations quite a stranger to letters, . . . which is a great hindrance to him in his reasonings uppon his Observations, for being ignorant of all other Mens thoughts, he is wholly trusting to his own.” Perhaps in this somewhat disparaging and patronizing description we have the very secret of Leeuwenhoek’s success, because as Leeuwenhoek himself said “novelties oft-times aren’t accepted, because men are apt to hold fast by what their Teachers have impressed on ‘em.”

Leeuwenhoek was thrilled by his own observations and he transmits his enthusiasm to the reader of his vivid descriptions. His discoveries are by no means limited to the field of bacteriology and protozoology, for his letters contain novel observations on matters zoological, botanical, chemical, physical, physiological and medical.

In his magnificently written and thoroughly documented biography, Dobell disperses the aura of fictional absurdities which has surrounded Leeuwenhoek’s accomplishments—and an even greater man emerges. In this respect, Dobell’s story of the life and accomplishments of Leeuwenhoek is the antithesis of the “debunking” trend of modern biographers.

The book is beautifully printed on fine stock, with a profusion of photolithographs and halftone plates. The education of every biologist is incomplete until he has absorbed its contents. But it is by no means a book for this small group of scientific workers alone. It is a story for all who wish to understand the true spirit of scientific inquiry, because the contents between its covers—set forth in a simple, beautiful and exciting manner—contain more of that spirit than any book I have ever read.

—WALTER M. SIMPSON

Streptococci in Relation to Man in Health and Disease. BY ANNA W. WILLIAMS. Pp. vi + 260, 1932, Baltimore, The Williams & Wilkins Company, \$5.00.

In this monograph the author, well known for her work on Streptococci, reviews the outstanding literature in the field. While her review is by no means as extensive as that published by the Pickett-Thompson Laboratory, it is far more readable and covers most of the important material published.

The book in part deals with the general characteristics of the group and their general manifestations and then specifically with local infections, elective localization, local immunity, erysipelas, scarlet fever, septic sore throat, the beta hemolytic group, the relation of streptococci to rheumatic fever, arthritis, measles, influenza, the common cold, poliomyelitis and encephalitis.

Much of the controversial part of the book centers around the work of Rosenow; evidently the author is not in agreement with him although she does not present sufficient evidence to match his thousands of experiments. These matters are forceably brought to the attention of the reader by such statements as "we all agree, except Rosenow and his associates" and, "the majority of us believe" which causes one to reflect on who "we all" really represent and how the "majority" was determined, if by actual count or by dead reckoning. The unbiased and critical reader will demand much more information than this book presents before supporting either side.

A table of 83 milk borne epidemics of scarlet fever and septic sore throat is especially valuable and well executed and the chapter on scarlet fever is very valuable and well done.

Two phases of the subject seem slighted: one, therapy in scarlet fever and what it has actually accomplished especially from a prophylactic standpoint, and second, the bacteriology of sub-acute bacterial endocarditis, which is covered in a scant page of text.

The book is certainly stimulating and although many will be disappointed in not finding more original work and more summaries and conclusions, great benefit will result from reading it.

THE BLOOD PICTURE IN PNEUMONIA*

WITH SPECIAL REFERENCE TO PATHOLOGICAL CHANGES IN THE NEUTROPHILS

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Blood changes in infections, especially pneumonia, have been observed for the past hundred years. The early investigators, working under technical difficulties, arrived at uniform results, namely:

- (1) Leukocytosis is present in the majority of pneumonia cases.
- (2) Leukopenia often indicates a fatal prognosis.
- (3) A possible relationship exists between the number of leukocytes, temperature, and area involved.
- (4) In the majority of cases, a transitory rise in the number of leukocytes appears during the early period of crisis, followed by a diminution.
- (5) Temperature reaches a normal level several days before the return of leukocytes to normal.
- (6) Delayed resolution and complications are accompanied by an increase in the number of leukocytes.
- (7) A steady rise in the number of leukocytes prognosticates a fatal ending.

Subsequent investigations were concerned with the variety of cellular reaction as well as with the leukocyte count in pneumonia. Türk,²⁶ Gaitskell⁷ and others observed the following changes:

- (1) Marked increase in the number of polymorphonuclear neutrophils; (in one case up to 96.5 per cent).
- (2) A diminution in the percentage of lymphocytes.
- (3) A disappearance or marked diminution in the eosinophil count.
- (4) A diminution in the percentage of polymorphonuclear neutrophils after crisis.
- (5) Appearance of myelocytes in many cases, most frequently at the crisis.

* Read before the Eleventh Annual Convention of the American Society of Clinical Pathologists, New Orleans, Louisiana, May 6-9, 1932.

Hickling¹⁰ found a transitory monocytosis after the crisis which bears no relation to prognosis.

Arneth² differentiated the polymorphonuclear neutrophil cell, according to the number of lobules in the nucleus, and suggested five divisions and many subdivisions. In cases of acute infection, he noted a marked increase in the number of cells, with fewer lobules in the polymorphonuclear neutrophils, indicating their increase by the term, "shift to the left." Due to the complexity of the Arneth formula, Schilling²⁵ suggested the following modification: (1) myelocytes (rarely found in normal blood), (2) young (up to 1 per cent), (3) staff or band (4 per cent), and (4) segmented leukocytes (67 per cent). In infections he found a marked increase chiefly in the staff (band) and young forms. Reznikoff²² in a study of seventy-eight cases of pneumonia found that, in 80 per cent of the patients who died from pneumonia, more than 30 per cent of the cells were immature polymorphonuclear neutrophils, which correspond to the staff (band) form of Schilling, and more than 40 per cent of all polymorphonuclear cells were immature; of those that recovered, 55 per cent showed more than 30 per cent of all cells to be immature, and 48 per cent showed more than 40 per cent of all polymorphonuclears to be immature.

Although the Arneth count has been somewhat modified by Schilling and by Cooke⁵ the present tendency is to adopt a still more simplified method, particularly one first proposed by Kothe¹² and emphasized recently by Pons and Krumbhaar,²⁰ who classified the polymorphonuclear neutrophils into segmented cells (average 70 per cent) and nonsegmented cells (average 4 to 10 per cent). Similarly, Farley, St. Clair and Reisinger⁶ classified the polymorphonuclear neutrophil cells into filamentous and nonfilamentous forms. This simpler classification is more readily understood than the more complicated hemograms of Arneth and of Schilling.

PATHOLOGICAL CYTOPLASMIC CHANGES

Important as the number of leukocytes and the differential count may be in determining the severity of the infection, it is, however, not diagnostic, as increases and changes may occur in

conditions other than infection. Certain studies on the toxic changes of the cytoplasm of the polymorphonuclear cells are equally if not more important. Cesaris-Demel⁴ described degenerative changes in polymorphonuclear cells in severe infections. Graham⁹ called attention to the changes in cytoplasm in severe infections, especially pneumonia, as shown by the diminution of the peroxidase granules. With the improvement of the patient these granules increase to their normal number. Using the ordinary blood stains (Jenner, Wright, Giemsa), Schleip,²⁴ Türk,²⁷ Naegeli,¹⁸ and Alder,¹ called attention to the deeply stained basophilic granules in severe infections. These are called toxic granules and are found in segmented as well as in nonsegmented polymorphonuclear neutrophils. Vacuolization is usually seen in segmented forms.

Pelgar¹⁹ called attention to another variety of cytoplasmic degeneration in which there is an absence of both granulation and peroxidase reaction, thus confirming Graham's findings.

Observation of the toxic granules in polymorphonuclear neutrophils is a valuable aid in the diagnosis and prognosis of cases of pneumonia. Pelgar observed the invariable presence of toxic granules in lobar pneumonia; their absence left no doubt as to the diagnosis. He cites an interesting case of so-called sepsis in a man sixty-one years of age whose chest was apparently negative. The blood count was: leukocytes, 33,000; nonsegmented polymorphonuclear neutrophils, 42 per cent; segmented polymorphonuclear neutrophils, 39 per cent; lymphocytes, 15 per cent; monocytes, 4 per cent; neutrophils with large toxic granules, 96 per cent. Pelgar considered the leukocyte count too high for sepsis, believing it to be typical of lobar pneumonia; he suggested the latter diagnosis despite the chest findings, and ventured a fatal prognosis. The signs of pneumonia only appeared three days prior to death. Postmortem examination revealed right upper lobar pneumonia with grey hepatization.

In bronchopneumonia, Pelgar found the toxic granules to be of smaller dimensions. The percentage of cells presenting these cytoplasmic changes was lower than in lobar pneumonia.

Mommsen¹⁵ also demonstrated the constant presence of toxic

granules in lobar pneumonia. He observed a rapid increase in the percentage of cells showing toxic granules; they reached their maximum (100 per cent) a few days after the crisis, and gradually returned to normal by the third or fourth week. When complications arose, particularly empyema, the number of toxic granules remained high (60 to 70 per cent) for weeks.

Gloor⁸ observed forty-seven cases of lobar pneumonia and noted the presence of toxic granules in all, but was unable to follow the quantitative variations in the toxic cells.

Mommsen^{16,17} reported additional findings on variations in the polymorphonuclear cells with toxic granules in eight cases of lobar pneumonia. Again, he found the maximal curve after the crisis. Even as late as the third week he observed that 30 to 40 per cent of polymorphonuclear cells may show toxic granulation. As the segmented and nonsegmented polymorphonuclear neutrophils usually showed the same percentage of toxic granules, he believed their presence could not be considered as an indication of immaturity of the cell. Furthermore, he believed that toxic granules are the result of physico-chemical alterations and that actual granular formation occurs extravascularly.

Bullock, Rosenbluth and Merkin³ examined the blood in 130 cases of pneumonia by means of the supravital technique (Sabin²³). The cells were found to be motile and, following serum treatment, presented no leukocytic changes before or after chills. The polymorphonuclear count had no relation to the recovery. Vacuolization and non-motility of the polymorphonuclear cells were found with greater frequency in patients who died.

IDENTIFICATION OF TOXIC GRANULES

The toxic changes manifest themselves by anisocytosis of polymorphonuclear neutrophils, slight degenerative alterations in the nucleus, and the presence of basophilic granulation in the cytoplasm (figures 1 and 2). These toxic or degenerative granules are of two kinds, small and large, and stain deeply basophilic with the usual blood stains. The small granules are distributed among the pinkish neutrophilic granules, and are present in milder forms of infection and during the period of convalescence.

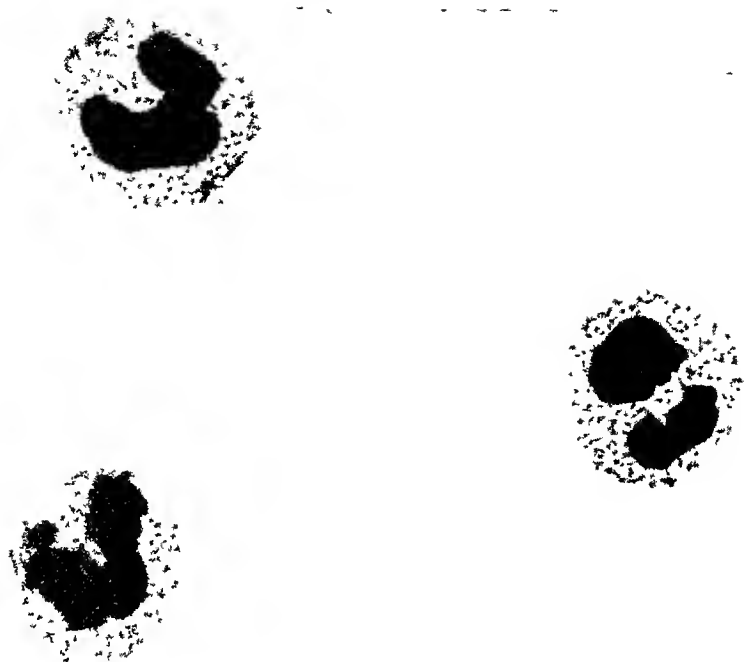


FIG. 1. TUBERCULOUS PLEURISY (CASE 40). NONSEGMENTED POLY-
MORPHONUCLEAR NEUTROPHILS WITHOUT TOXIC GRANULES ($\times 1500$)



FIG. 2. ACUTE LOBAR PNEUMONIA (JENNER-GIEMSA STAIN) (CASE 14). ONE
SEGMENTED AND TWO NONSEGMENTED POLYMORPHONUCLEAR NEUTRO-
PHILS WITH TOXIC GRANULATION ($\times 1500$)

The larger toxic granules appear in cells devoid of pinkish neutrophilic cytoplasm, and are present in severe infections. Lighter bluish cytoplasmic masses may likewise be present in severe infections; these correspond to the Doehle bodies, first described in scarlet fever.

Due to variations in the stains, control smears are important in the differentiation of these granules. Such control smears can be made by using one-half the slide for the patient's blood and the other half for normal blood.

The most practical stain is that of Wright, with the use of a buffer solution (pH 6.4). Best results are obtained, however, with the Jenner-Giemsa stain, using a buffer solution, (pH 6.8 to 7.0). Staining is performed as follows:

- (1) Jenner stain (National Aniline & Chemical Company) is used, sufficient to cover the slide for one minute.
- (2) Dilute with buffered distilled water (pH 6.8 to 7.0) for three minutes.
- (3) Wash off diluted stain with buffered solution.
- (4) Cover slide with Giemsa Stain (R. A. L.), diluted with one drop to 1 cc. of buffered distilled water.
- (5) Wash and allow to dry in the air.

The buffer solution for the Jenner-Giemsa stain can be prepared by diluting 0.5 cc. to 3.0 cc. of M/15 Na_2HPO_4 to 1000 cc. of distilled water; the amount added varies with the original pH of the distilled water.

For Wright's stain, the buffer solution recommended by McJunkin¹⁴ is preferable. This buffer is prepared by dissolving 6.63 grams of Merck's monopotassium phosphate and 2.56 grams of Merck's anhydrous disodium phosphate in 1 liter of distilled water (add about 1 cc. of chloroform).

A comparative study of the number of polymorphonuclear cells with toxic granules (Figure 2) with the total number of polymorphonuclear cells offers a guide to the progress of infection. The degenerative index may be obtained from this ratio:

$$\frac{\text{number of toxic polymorphonuclear cells}}{\text{total number of polymorphonuclear cells}}$$

A rise in the index to 100 per cent, with the appearance of large toxic granules, indicates a severe or widespread suppurative infection. A gradual or rapid diminution in the index indicates a good prognosis (Kugel and Rosenthal¹⁵).

Complete blood counts are also made at frequent intervals using a modified hemogram. (See example as illustrated in table 1.)

CASES ILLUSTRATING RECOVERY FROM PNEUMONIA

The following selected cases (table 2) illustrate some of the variations in the blood picture in mild, severe and complicated lobar pneumonia.

Case 10 (Mild type): Patient, aged fourteen years, was admitted to the hospital a year previously for asthma and readmitted with a three-day history of pain in chest, cough, and fever. Signs of lobar pneumonia were present in

TABLE 1

MODIFIED HEMOGRAM

Patient: C. B. (Case 29). Ward: E. Date: 2-13-31.

Diagnosis: Lobar pneumonia.

Hemoglobin: 85 per cent.

Erythrocytes: 4,550,000. Platelets: 760,000. Leukocytes: 31,000.

| | TOTAL | NORMAL | TOXIC |
|--------------------------------------|----------|--------|----------|
| | per cent | | per cent |
| Differential count: | | | |
| Polymorphonuclears, nonsegmented.... | 43 | 0 | 43 |
| Polymorphonuclears, segmented..... | 41 | 0 | 41 |
| Polymorphonuclears, eosinophile..... | 0 | | |
| Polymorphonuclears, basophile..... | 0 | | |
| Lymphocytes..... | 12 | | |
| Monocytes..... | 1 | | |
| Other cells: | | | |
| Myelocytes..... | 3 | | |

Degenerative index: 100.

Clinical data: acute lobar pneumonia thirteenth day of illness. Died on the fourteenth day of illness.

the right, middle and right lower lobes (confirmed by x-ray). The temperature (105°) dropped in four days by crisis.

The blood picture on admission (4th day of illness) showed a moderate leukocytosis and increase of the nonsegmented polymorphonuclear neutrophils (38 per cent), and 51 per cent segmented neutrophils. The degenerative index was 4; this increased daily to 86 after the crisis and returned to normal on the 26th day of illness. A post-critical monocytosis was present in this particular case. The hemoglobin, erythrocytes and blood-platelets were normal.

Case 18 (Severe type): A middle-aged female hospital employee (table 2) with a history of arthritis, complained of chilliness, cough and fever for two days. Dullness was present in the right base posteriorly and in the right

and left apices. X-rays showed bilateral upper lobar pneumonia. The temperature was 103° on admission and dropped by crisis on the 13th day of illness.

Blood Picture (Chart 1): The hemoglobin, erythrocytes and platelets were normal throughout the course. There was a striking leukocytosis of 50,000 to 87,000 before the crisis. The nonsegmented polymorphonuclear neutrophils (71 per cent) fell rapidly to normal by the 17th day while the segmented neutrophils and lymphocytes increased to a normal percentage during the same period. The degenerative index did not run parallel to the nonsegmented cells. It increased rapidly before the crisis to 100 (indicating that 100 per cent

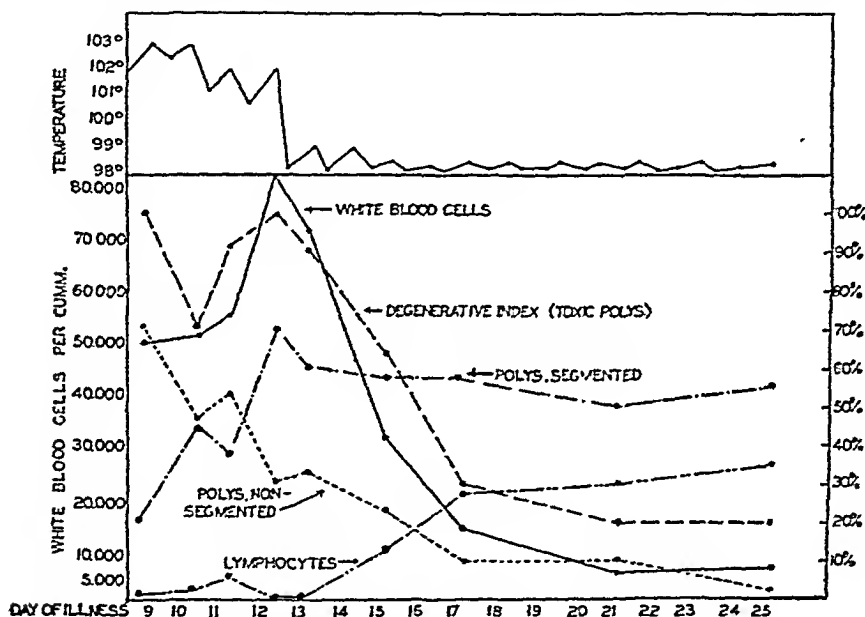


CHART 1. ACUTE LOBAR PNEUMONIA (CASE 18)

of the neutrophils contained toxic granules) and then diminished rapidly to 20 on the 25th day of the illness, when the leukocyte count was 7,900. Notwithstanding the fact that the leukocyte count was normal, evidence of resolution was demonstrated by the presence of toxic cytoplasmic changes in the neutrophils. The myelocytes and monocytes were not increased during the period of observation.

Case 14 (table 2): Pneumonia complicated by empyema. A housewife, aged thirty-one years, developed a cough, bloody sputum, fever, chill, and pain in the right chest five days before admission to the hospital on February 12th (table 2).

The physical examination revealed a lobar pneumonia of the right upper

and lower lobes. Several days later the left lower lobe became involved. The condition had a very stormy course.

The blood changes were typical of lobar pneumonia: moderate leukocytosis (18,000-26,000) and marked increase of the nonsegmented neutrophils to 66

TABLE 2
LOBAR PNEUMONIA—RECOVERED CASES

| CARD NUMBER | AGE | DAY OF ILLNESS* | HEMOGLOBIN | LEUKOCYTES | POLYMORPHO- NUCLEAR NEUTROPHILS | | LYMPHOCYTES | MONOCYTES | MYELOCYTES | DEGENERATIVE INDEX | PNEUMOCOCCUS | |
|-------------|-----|-----------------|------------|------------|---------------------------------------|-----------|-------------|-----------|------------|--------------------|---------------|---------------|
| | | | | | Non-segmented | Segmented | | | | | Blood culture | Sputum typing |
| | | | per cent | | per cent | per cent | per cent | per cent | per cent | per cent | | |
| 1 | 16 | 1 | 80 | 16,500 | 16 | 70 | 11 | 3 | 0 | 0* | Negative | IV |
| 2 | 40 | 2 | 85 | 19,600 | 47 | 41 | 10 | 1 | 0 | 57 | Type II | |
| 3 | 22 | 2 | 75 | 15,000 | 43 | 48 | 6 | 3 | 0 | 9 | Negative | |
| 4 | 22 | 2 | 85 | 17,000 | 56 | 35 | 3 | 1 | 0 | 49 | Type I | |
| 5 | 33 | 2 | 80 | 7,200 | 1 | 58 | 28 | 9 | 0 | 34 | Negative | IV |
| 6 | 37 | 3 | 75 | 25,000 | 47 | 45 | 2 | 4 | 2 | 78 | Negative | IV |
| 7 | 30 | 3 | 90 | 20,800 | 60 | 23 | 10 | 3 | 4 | 90 | Negative | Negative |
| 8 | 8 | 3 | 72 | 22,400 | 56 | 23 | 12 | 8 | 0 | 100 | Negative | Negative |
| 9 | 25 | 4 | 80 | 9,500 | 37 | 51 | 4 | 2 | 6 | 72 | Type IV | IV |
| 10 | 14 | 4 | 88 | 16,000 | 38 | 51 | 5 | 6 | 0 | 4 | Negative | III |
| 11 | 35 | 5 | 80 | 8,500 | 34 | 38 | 16 | 12 | 0 | 33 | Negative | III |
| 12 | 22 | 6 | 90 | 13,000 | 54 | 25 | 13 | 5 | 0 | 100 | Type I | I |
| 13 | 27 | 6 | 75 | 22,000 | 41 | 46 | 7 | 6 | 0 | 31 | Negative | IV |
| 14 | 31 | 6 | 81 | 18,500 | 39 | 58 | 2 | 1 | 2 | 92 | Negative | II |
| 15 | 39 | 7 | 86 | 16,600 | 62 | 17 | 17 | 2 | 0 | 98 | | IV |
| 16 | 36 | 7 | 77 | 8,000 | 34 | 38 | 21 | 3 | 2 | 80 | Negative | III |
| 17 | 36 | 8 | 85 | 27,000 | 50 | 39 | 4 | 2 | 3 | 75 | Negative | Negative |
| 18 | 40 | 9 | 80 | 51,600 | 71 | 21 | 1 | 3 | 2 | 100 | Negative | |
| 19 | 55 | 9 | 78 | 14,000 | 64 | 30 | 6 | 0 | 0 | 32 | | |
| 20 | 44 | 9 | 72 | 23,100 | 58 | 29 | 3 | 0 | 0 | 94 | Negative | I |
| 21 | 23 | 9 | 80 | 11,200 | 46 | 24 | 10 | 8 | 10 | 84 | | |
| 22 | 46 | 12 | 75 | 7,500 | 4 | 71 | 12 | 6 | 1 | 86 | Negative | I |
| 23 | 45 | 14 | 70 | 10,000 | 32 | 50 | 13 | 4 | 0 | 72 | Negative | Negative |
| 24 | 17 | 31 | 85 | 12,600 | 8 | 51 | 30 | 6 | 0 | 60 | Negative | Negative |

* On admission to the hospital.

per cent, occasional myelocytes and the appearance of post-critical monocytosis (13 per cent). The degenerative index was high at first (92) and gradually returned to normal on the 45th day of her illness (March 11th), although there had been an increase in the number of leukocytes to 140,000 (March 9th).

On March 17 (51st day of illness), there was a marked rise in the degenerative index (92). An empyema on the left side was found two days later and thoractomy was performed, 1,000 cc. of pus (subsequently shown pneumococcus III on culture) being evacuated. The patient made an uneventful recovery.

Case 2: Atypical blood findings in a patient with pneumonia, complicated by empyema. A man, aged forty years, addicted to the use of alcohol for twenty years, was admitted with sharp pains in the right chest. Bronchial breathing and egophony were heard at the right base. The roentgen examination confirmed the diagnosis of a right lower lobar pneumonia. The patient was fairly comfortable, the temperature was subfebrile, never above 101. The blood culture was positive for pneumococcus type II. No sputum could be obtained for typing. The blood picture showed a leukocytosis (19,600-31,900) and only a moderately high degenerative index (57). The non-segmented neutrophils were as high as 47 per cent at first (2nd day of illness), but returned to normal by the 13th day. At that time there was still a slight leukocytosis, a normal differential count and absence of toxic granulation. The temperature remained low (100). However, signs in the chest persisted and, later, evidence of a marked accumulation of fluid was found. The right side of the chest was aspirated and the fluid proved to be thick pus. A thoractomy was performed a few days later. The blood picture (table 4) remained normal except for a slight leukocytosis (15,400) and slight increase of the toxic cells (degenerative index (15)).

The deviations from the normal reaction in this patient were: (1) subfebrile course although bacteremia was present, (2) moderate leukocytosis with lower trend in the degenerative index, (3) leukocytosis without increase of the non-segmented polynuclears with the appearance of a severe complication (empyema), (4) absence of toxic granulations in the neutrophils when the empyema was found.

RÉSUMÉ OF OBSERVATIONS ON RECOVERED CASES

Observations on the blood picture during the course of twenty-four cases (table 2) of lobar pneumonia, with recovery disclose the following significant facts:

(1) The hemoglobin, erythrocytes and platelets showed slight variations. The lowest hemoglobin was 70 per cent, the average more than 80 per cent. The blood platelets were uniformly normal. In only two cases were the platelets less than 200,000 per cubic millimeter (140,000 and 150,000).

(2) The leukocytes varied from 7,200 (table 2, case 5) to 51,600 (table 2, case 18). There was no uniformity in their varia-

tions before or after the crisis; there was either an increase or decrease.

The nonsegmented polymorphonuclear neutrophils were increased in all except three cases from 16 to 71 per cent. In three cases of lobar pneumonia, confirmed by x-rays, the nonsegmented polymorphonuclear neutrophils were normal on the patient's admission (table 2, cases 5, 22, 24). In these cases, however, there was a rapid increase before the crisis.

The lymphocytes were markedly diminished. The monocytes were either absent or normal and only slightly increased (8 to 12 per cent) in three cases (table 2, cases 5, 11, 21). A post-critical monocytosis was exceptional.

(3) The degenerative index (the percentage of polymorphonuclear neutrophils with toxic granules) was found to be very high (31 to 100 per cent) with three exceptions: Case 1 represented an atypical one-day pneumonia (Woiliez Disease) confirmed by roentgenogram. Some consider this condition a result of pulmonary congestion and not a true pneumonia (Ramond²¹). Case 3 and case 10 (table 2) were mild cases, but the degenerative index rose rapidly to 70 and 86 respectively at the crisis and returned to normal by the end of the third week. The downward trend of the degenerative index is a good prognostic sign.

(4) In patients addicted to alcohol the blood findings were less typical. The degenerative index was comparatively lower. During severe complications, such as empyema, the nonsegmented polymorphonuclear neutrophils and the degenerative index may be normal.

FATAL CASES OF PNEUMONIA (TABLE 3)

The blood changes in fatal pneumonia at the onset showed variations, somewhat similar to those of the recovered cases. A moderately severe anemia was present in three cases only. The leukocytes varied from 5,100 to 36,000; in four cases (table 3, cases 25, 27, 31 and 34) there was a subsequent marked leukopenia which may be considered an unfavorable sign. The degenerative index was invariably 100 during the course of observation, in all but one case. In this patient (table 3, case 34), the pneu-

monia was of a chronic type. The patient was quite toxic and a leukopenic tendency was present, the highest degenerative index being 37. The persistence of the high degenerative index (100) in these cases may be considered a bad prognostic sign, and as such is more reliable than the variations in the nonsegmented polymorphonuclear neutrophils.

TABLE 3
LOBAR PNEUMONIA—FATAL CASES

| CASE NUMBER | AGE | DAY OF ILLNESS* | HEMOGLOBIN | LEUKOCYTES | POLYMORPHO- NUCLEAR NEUTROPHILS | | LYMPHOCYTES | MONOCYTES | MYELOCYTES | DEGENERATIVE INDEX | PNEUMOCOCCUS | |
|-------------|-----|-----------------|------------|------------|---------------------------------------|-----------|-------------|-----------|------------|--------------------|---------------|---------------|
| | | | | | Non-segmented | Segmented | | | | | Blood culture | Sputum typing |
| | | | per cent | | per cent | per cent | per cent | per cent | per cent | | | |
| 25 | 40 | 3 | 82 | 8,400 | 72 | 15 | 4 | 2 | 0 | 100 | Negative | |
| 26 | 45 | 5 | 88 | 20,400 | 51 | 43 | 4 | 2 | 0 | 100 | | |
| 27 | 37 | 7 | 79 | 8,000 | 20 | 59 | 18 | 3 | 0 | 100 | Type IV | Negative |
| 28 | 80 | 7 | 80 | 21,800 | 44 | 42 | 13 | 1 | 0 | 100 | Type III | Negative |
| 29 | 45 | 10 | 85 | 31,000 | 43 | 41 | 12 | 1 | 3 | 100 | | |
| 30 | 57 | 11 | 86 | 24,000 | 28 | 65 | 6 | 1 | 0 | 100 | Negative | I |
| 31 | 5 | 12 | 65 | 5,100 | 48 | 15 | 15 | 13 | 9 | 100 | | |
| 32 | 40 | 13 | 55 | 36,000 | 55 | 37 | 6 | 1 | 0 | 100 | Type IV | |
| 33 | 28 | 14 | 85 | 18,850 | 26 | 56 | 16 | 2 | 0 | 100 | Negative | Negative |
| 34 | 40 | 14 | 75 | 6,100 | 37 | 45 | 16 | 2 | 0 | 47 | Negative | IV |

* On admission to the hospital.

CASES SIMULATING PNEUMONIA

The constant presence of toxic granulation in the polymorphonuclear neutrophils in pneumonia is an important diagnostic criterion in the differentiation of such cases from those simulating pneumonia. The following case will serve as an example:

Case 40: A man, aged forty-two years, was admitted to the hospital complaining of a severe chill which had lasted for ten minutes, and a cough. His temperature was 103°.

Dullness was present in the right chest posterior to the angle of the scapula. The heart was not displaced. The blood culture was negative. Roentgenogram revealed a shadow to the right of the heart which seemed to be a peri-

mediastinal pleural effusion. The blood examination showed a leukocyte count of 6000 and 31 per cent of nonsegmented neutrophils. During his stay in the hospital, toxic granules were not found (fig. 1) so that pneumonia could be definitely ruled out. Later observations indicated that the patient was suffering from a tuberculous pleural effusion.

The reverse is also important, as seen recently in a child who was brought to the hospital for an appendectomy. There were some abdominal signs, and even slight tenderness in the right lower quadrant. The blood examination revealed the presence of toxic granulations which led to a suspicion of pneumonia. The surgeon was advised to delay operation until a roentgenogram of the chest was done which confirmed the diagnosis of pneumonia.

Other patients were admitted to the hospital with a tentative diagnosis of pneumonia. The absence of the toxic granules for the first few days, or their marked diminution, helped to rule out this particular diagnosis.

Recently, patients have been observed with cardiac decompensation and pulmonary signs. The symptoms as well as the physical signs in the chest may closely resemble pneumonia. The presence of toxic granules is definitely indicative of pneumonia; their absence points to congestion, infarction and other conditions.

DISCUSSION

Several factors must be considered with respect to the blood changes in lobar pneumonia, and their relation to diagnosis and prognosis.

The number of leukocytes showed very marked variation from 4,000 to 144,000, the latter count recently observed in a child. In our series the highest in an adult was 85,000 (chart 1, case 18).

The differential count showed a marked variation in the number of nonsegmented polymorphonuclear neutrophils from 4 to 72 per cent.

Slight secondary anemia was present in about 33 per cent of the cases and more marked anemia in 25 per cent of those that were fatal. These changes are present in other types of infection and are not particularly diagnostic of pneumonia. They seem to indicate the presence of a severe infection.

Cytoplasmic changes with the constant appearance of toxic granules are present in lobar pneumonia and are usually absent in polymorphonuclear cells in other conditions simulating pneumonia, such as pulmonary congestion, infarcts of the lung in cardiac cases, pleurisy with effusion, lung abscess, rheumatic pneumonia, hydrothorax and pulmonary tuberculosis. In pneumonia the toxic granules appear rather early and have been found on the second day in a fairly large number of polymorphonuclear cells. As first pointed out by Pelgar, Hittmaier,¹¹ Mommsen and Gloor, the appearance of the pathological cytoplasmic changes may be present in all polymorphonuclear cells a few days after the onset of the disease, or they may reach their maximum prior to, or immediately after, the crisis. The percentage of polymorphonuclear cells showing toxic granules, that is, a degenerative index (Kugel and Rosenthal) may be followed throughout the course of pneumonia. This index remains fairly high in some cases for an invariable period, but usually the percentage of cells showing toxic granules rapidly diminishes within twelve to fourteen days after the crisis. However, as late as the third or fourth week, there may be a persistence of smaller granules in polymorphonuclear cells, as reported previously by Mommsen.

The only exception to these results occurred in two patients who were addicted to the use of alcohol. In both, the degenerative index had a tendency to be lower (the highest being 63), returning rapidly to normal as the patient improved.

The factors involved in prognosis are the anemia, the number of leukocytes and the percentage of nonsegmented neutrophils and the degenerative index. The question arises whether the prognosis can be stated definitely from the first examination of the blood. This is sometimes possible when a marked leukopenia is present. Apart from the occurrence of the leukopenia, however, prognosis depends upon the trend of symptoms, the extent of involvement in the lung and the trend of the blood changes.

A severe anemia or a marked increase in the number of nonsegmented cells (above 65 per cent) may presage a fatal outcome. The presence of bacteriemia is not indicative of a bad prognosis, although in the recovered cases here reported, a blood culture was

positive in 20 per cent of the cases, and in the fatal cases, in 33 per cent.

Follow-up of the degenerative index is important in prognosis. A rapidly diminishing degenerative index presages a rapid recovery; gradual diminution of the degenerative index keeps pace with gradual recovery. The persistence of a high degenerative index indicates either a fatal outcome or the presence of complications. A secondary rise in the degenerative index during convalescence is indicative of complications, usually empyema.

CONCLUSIONS

(1) In addition to noting ordinary blood changes, observations on pathological cytoplasmic alterations (toxic granules) are a valuable aid in the diagnosis and prognosis of cases of pneumonia.

(2) Pathological cytoplasmic alterations (toxic granules) are present in all cases of lobar pneumonia, are diminished in bronchopneumonia, and usually are absent in conditions simulating pneumonia.

(3) In recovered cases, the degenerative index—that is, the percentage of cells showing toxic changes—rises rapidly (not infrequently to 100) either prior to, or immediately after, the crisis and persists much longer than the symptoms, that is, to the third or fourth week.

(4) The degenerative index is lower in alcoholic patients suffering from pneumonia.

(5) In fatal pneumonia the degenerative index is frequently 100; its persistence at that level presages a fatal prognosis.

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THE AUTOPSY*

AN OUTLINE OF THE PROBLEM

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In reviewing the literature since 1913 dealing with autopsies, I found 288 articles and editorials in the various American medical and hospital publications. It is possible that some, though probably only very few, were missed. In spite of all this, I feel it may be worth while to review briefly the results of the autopsy movement in this country, and because I believe that the limited success or relative failure, as you wish to call it, is due to a basically wrong conception of the problem involved. To prove that will be the purpose of my presentation.

To begin with, let us look back and review briefly the 288 articles and editorials. The writers are, in the order of frequency, pathologists, physicians, both general practitioners and specialists, hospital superintendents, internes, a few undertakers and one rabbi. They touch upon a variety of aspects of the subject with natural and unavoidable repetitions. The essence of what is expressed and advocated in that veritable library of publications could readily be boiled down to a few brief paragraphs.

The basic question of whether autopsies are at all desirable is quite naturally affirmed by all but one of the contributors, the dissenting voice coming from an anonymous editorial writer of an undertakers' publication in Chicago. One of the medical writers, while advocating autopsies very warmly, is inclined to set an age limit by stating that they ought to be done on persons dying before the age of sixty-five. No reason is given for such a limitation; possibly the author does not care much what happens

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after one is sixty-five. The training of internes, the post-graduate education of the practicing physicians, the advance of medical knowledge, and the establishing of true causes of death and hence placing vital statistics on a sound basis are quoted as the main arguments in favor of autopsies. Having thus established the desirability of autopsies, the authors suggest innumerable schemes which are supposed to enable physicians to obtain permissions for autopsies from the relatives of the deceased. All of those schemes are inspired by the best intentions and when applied are more or less successful. Some of them are unmistakably influenced by the methods of up-to-date salesmanship, as practiced in modern business, but not infrequently the obtaining of an autopsy permit depends on such methods. Those who have personally tried to get permissions for autopsies will probably agree with me. I believe that to this phase, the technic of getting permits for autopsies, hardly anything can be added.

Various religious objections have been thoroughly analyzed and suggestions offered as to how to meet them. A great deal of consideration was given to difficulties arising from lack of coöperation on the part of the undertakers. Their justifiable complaints were presented and the pathologists urged to meet their demands. If we add to the above, suggestions for educational propaganda among physicians as well as among the laity on the value of autopsies we have practically all that is essential which has appeared in the literature on this subject during the last eighteen years.

What are the results? It would be very difficult to estimate, even with an approximate accuracy, the results of this campaign for postmortem examinations. Unfortunately, figures are available only in a few larger cities; in Chicago the Institute of Medicine has published annually, since 1919, a very accurate report. Less accurate data are available for some of the other large cities. There is no doubt that, compared with the conditions which prevailed in 1913, the number of permitted autopsies has greatly increased. Despite the increase, Karsner¹ estimated, in 1927, that less than 0.7 per cent of the country's dead was examined after death. Even if we assume that that figure has since

doubled, it would mean that in more than 98 per cent of deaths in this country there is reason for considerable doubt as to the correctness of the cause of death as given on the official death certificate. Very instructive is the comparison of autopsy percentages for the years 1926 and 1930 in the hospitals approved by the American Medical Association for the training of internes.

An analysis of the figures in the table shows in a rather convincing manner that the increase in the percentages for 1930 in the approved hospitals was the result of the ruling of the American Medical Association. There was no increase in the number of hospitals having 70 per cent or more, or in the whole group of those reporting 50 per cent or less. However in the latter group the number of those reporting 20 to 49 per cent has doubled, while those below 20 per cent have become fewer.

While it is true that autopsies are being performed in non-approved hospitals, as well as in those on the approved list, the efforts are probably greater in those on the approved list and they must be considered as representing the best, most progressive hospitals in the country. There can be no doubt that the increase in the number of autopsies within the groups having less than 50 per cent is a direct result of the pressure by the American Medical Association. Many hospitals are having considerable difficulty to live up to the 15 per cent requirement set by the American Medical Association for hospitals approved for the training of internes.

All this, while it would mean a considerable progress, illustrates that the success is only relative. Furthermore, we are well aware that the efforts expended to achieve the above results are really extraordinary. While in some larger institutions, particularly in teaching hospitals, after the establishment of a routine and organization striving to obtain autopsies, the effort does not seem to be excessive, in smaller, voluntary hospitals a huge amount of energy is being expended. Turner⁴ estimated the cost of an autopsy at the Mount Sinai Hospital in New York to be \$92.40; he did not include the time and energy of the people engaged in obtaining permissions. It is true that this effort cannot be very easily expressed in dollars and cents. However, the person doing it must be endowed with high grade personality and intelligence,

and his time is valuable. In some hospitals the superintendent, the attending physicians and the internes are spending hours, on occasions, trying to obtain permissions. I am convinced that this effort, expressed in figures, would sometimes come very close to the other expenses involved. Thus, considering all the factors, I believe, and I know that many feel that way, that the result can hardly be called a great success.

Therefore, while we may disagree as to the opinion of how great or how small is the success of the autopsy movement in this country, there is hardly anybody who is optimistic when considering the hospitals as a whole, and not only the large institutions connected with medical schools. Even assuming that the results in hospitals connected with teaching institutions are satisfactory, the fact that they constitute only about 300 of the total of 6719 hospitals does not change the impression that, generally speaking, the number of autopsies is deplorably low.

How are the relatively meager results to be explained? I believe that there are at least two major causes: One of them was discussed in the literature in recent years; the other, the much more important cause, was, to my knowledge, not sufficiently emphasized. I shall begin with the second cause. There is a fundamental error in the general conception of autopsies, particularly as far as the obligations of the medical profession are concerned. I think that it would be best to illustrate this with an example. Suppose that years ago, when, as a result of advanced medical knowledge it became evident that sanitary measures of various kinds, like the disposal of sewage, disinfection, et cetera, must be employed for the control of epidemics, the task of execution and enforcement had been left entirely to the medical profession without the help of proper laws and of authority of government. What would have been the outcome of such an attempt? What would happen if the physicians would have to get the permission of the people to have them quarantined? Is there a doubt that the movement would have been doomed to dismal failure? To me, there is no difference between the importance of autopsies in all its phases and the problem of sanitation. It is very unfortunate that the medical profession took upon its

own shoulders or had forced upon itself the responsibility of handling that problem.

We are sometimes generous, in fact too generous, in speaking of the conditions in Germany and Austria in that respect, and credit

TABLE 1*

THE EFFECT OF THE MINIMAL AUTOPSY REQUIREMENT OF THE AMERICAN MEDICAL ASSOCIATION UPON THE AUTOPSY PERCENTAGES IN APPROVED HOSPITALS

| PERCENTAGE OF AUTOPSIES | NUMBER OF HOSPITALS | |
|-------------------------|---------------------|------|
| | 1925 | 1930 |
| 100-90 | 3 | 0 |
| 89-80 | 2 | 4 |
| 79-70 | 9 | 10 |
| 69-60 | 13 | 26 |
| 59-50 | 8 | 26 |
| 49-40 | 26 | 59 |
| 39-30 | 42 | 87 |
| 29-20 | 91 | 184 |
| 19-15 | 55 | 143 |
| 14-10 | 107 | 45 |
| 9-5 | 120 | 18 |
| 4-1 | 80 | 2 |
| None | 5 | 1 |
| No record | 3 | |
| Not reporting | 14 | 59 |
| Totals | 578 | 664 |
| 70 or over | 14 | 14 |
| 50 or over | 35 | 66 |
| Below 50 | 526 | 539 |
| 20-49 | 159 | 330 |
| 5-19 | 282 | 206 |

* Condensed from material contained in the eighth and tenth annual presentations of hospital data by the Council on Medical Education and Hospitals of the American Medical Association. Jour. Am. Med. Assn., 92: 1043-1127. 1929 and 96: 1009-1086. 1931.

the medical profession in those countries with the merit of their relatively great success, but we do not realize that there the conditions with regard to autopsies are regulated by laws or by established customs. The medical profession in this country

cannot solve the problem of autopsies any more than it could have been solved by the medical profession of any other country under the same circumstances. No greater handicap could have been put in the way of the movement. I do not know of a more difficult task than is, in some cases, the obtaining of a permission for an autopsy.

In most parts of Germany the custom is that the relatives are notified of the patient's death and have the privilege of protesting against an autopsy during the first twenty-four hours. In Austria, the autopsy in public hospitals is made obligatory by the law. In this country we must approach the relatives for a permission at a most inopportune time. I cannot help feeling, and many who have experienced it will agree with me, that there is a certain cruelty in such an attempt, even when we are dealing with broad-minded people, and particularly so in the case of emotional persons. And who is not emotional at a time when he loses a dear one? It is true that, as conditions are in this country, it almost appears visionary to think of a time when a law will be passed making the performance of autopsies easier; we are afraid to think of the difficulties with which the enforcement of unpopular laws is connected. But, on the other hand, certain sanitary laws are not at all popular and nobody thinks of asking individuals whether they approve of the necessity of being quarantined in the case of epidemic diseases. I used to be very pessimistic in this respect, and I thought that everything else will have to be tried before we think of legislation in regard to autopsies. I believe now that all other attempts will not lead to a great success. It seems to me that a consideration of the development of sanitary laws in this country points toward a possibility of improvement in the autopsy situation.

Is there a doubt in the mind of any one that the autopsy is a public health problem of the greatest importance? The progress of scientific medicine, the question of heredity of diseases are all intimately connected with the autopsy. If a clinical statement as to the cause of death requires the final check-up of an autopsy to be scientifically correct and unassailable, as is generally agreed, then in over 98 per cent of cases the statements as to the causes of

death are not defensible scientifically, and our vital statistics are of very little value. You know how many conclusions of theoretical and practical nature are drawn from vital statistics. To quote only one example: Premiums for life insurance are based on calculations and conclusions derived from the vital statistics. These calculations cannot be correct because the facts on which they are based are not correct.

Now, last but not least, we are coming to the effect of autopsies upon the professional efficiency of the physician. All agree that the training in diagnosing diseases acquired by the medical student in school cannot possibly make him an accomplished diagnostician, and that he has to remain a student of medicine all his life. Everybody admits that the best way for the physician to perfect his diagnostic efficiency is by evaluating his diagnoses and the results of his treatment at the autopsies of those of his patients who had the misfortune to die. The need of experienced physicians is unquestionably of great importance for public health, no less so than medical schools, and still the maintenance of medical schools is not left entirely to the medical profession. What have public health authorities in this country and the government done to secure for the people the undisputed and scientifically established benefits of autopsies? Not one thing. Whatever laws there are, they are directed against and not in favor of autopsies. I do not know exactly, and until now I had neither the time nor the opportunity to study how the sanitary laws in this country were enacted, but I doubt very much whether it was deemed necessary and advisable to educate the masses of people to a point when they themselves demanded that such laws be passed and enforced. The medical profession naturally had to act in an advisory capacity in matters of purely medical nature but was hardly expected to bear the whole brunt of the movement which proved to be of the greatest benefit to humanity. I believe that it will have to be in a similar way that a change in the attitude of the public health authorities will take place relative to autopsies. The sooner the authorities concerned with public health matters realize that, the sooner something will be done. It was simply taken for granted that the problem of autopsies

would be solved exclusively by the medical profession without the help of other agencies and against terrific odds, and the medical profession, already burdened with more difficult tasks than any other, had good-naturedly taken it upon itself. Failure was unavoidable.

I am unfit to make any definite practical suggestions, and have only a few vague ideas on how I visualize the possible future development. The first thing that, in my humble opinion, will have to be done is to have pathologists, statisticians, those specializing in public health matters, and whoever else may feel qualified and inclined prove or disprove that the autopsy is primarily a public health problem concerning the welfare of present and future generations, and that its domain is such that the benefits derived from it cannot be arrived at by any other means. Once this problem is settled, and I believe that it will be settled in the positive sense, then there should not be undue difficulty in changing the entire situation. The National Council on Autopsies now in the process of formation, following a suggestion of the American Hospital Association, may be the logical organization to include into its program the proposals which are here suggested.

I wanted to show in the preceding paragraphs what, in my opinion, was the basic mistake in the concept of autopsies and why the very commendable, though costly, movement to promote autopsies in this country brought only relatively meager results in the last few decades, certainly entirely out of proportion to the amount of effort expended.

I am afraid that objections will be raised that the concept as presented is an impractical dream, that it cannot be realized, and that it may have a depressing and discouraging effect upon the movement, in that it will be employed as an excuse for not attempting to try obtaining autopsies by the means available. Such objections may be justified to some extent but that is no reason for keeping one's eyes closed to the real issue.

I wonder how it can be denied that, as matters stand now, it is generally agreed that the autopsies concern nobody else but the medical profession and the hospitals. Who doubts that by the discarding of such a false and dangerous idea a great deal of good

will ultimately be accomplished? I do not wish to appear visionary and impractical and should not like to be misunderstood. I am fully aware that it may take a long time before anything practical will result. Till then there is a great deal to be done, with the means now on hand, to strive towards an improvement of the prevailing situation.

In this connection I should like to discuss the second major reason for the rather unsatisfactory results of the autopsy movement. The second cause is that the medical profession, while taking upon itself the selling to the public of the idea of autopsies, did not sell it, first of all, to its own members. Thus the fundamental condition was not fulfilled, without which a success could not possibly be expected. Those who doubt the correctness of the last statement are not acquainted with the real situation. Among others, Robertson² and Lynch², have emphatically expressed this very thought; in fact, it is impossible to improve upon their analyses of this phase. To be even partially successful in the attempt of giving to the people the benefits of autopsies we certainly will have to sell the idea of autopsies, first of all, to our own colleagues, and this duty will have to evolve primarily upon the pathologists of this country. There is one argument in favor of autopsies which, if properly emphasized, will appeal to the medical practitioner more than all the others taken together, and that is that he can never expect to be a good physician unless he follows his patients into the autopsy room. This argument is much more appealing than the importance of autopsies for medical progress, et cetera. It seems strange, but nevertheless true, that a good portion of the medical profession has not come to a full realization of the fact that the autopsy is the most important of all means by which the physician can advance his diagnostic efficiency. This propaganda will not be successful if limited only to theoretical discussions. It is up to the pathologists of this country to prove the advantages of autopsies to the medical practitioners.

Some pathologists will have to lay greater emphasis upon their autopsy work. They will have to perform the autopsy and present the findings in a less detached and academic way, and will have to pay particular attention to the correlation of clinical and

anatomical findings. To do so they will have to acquaint themselves with the clinical point of view; they will have to know very thoroughly the clinical history of the patient, because only then will they be able to point out those changes in which the clinician is particularly interested. One can, after some practice, make the autopsy findings of much greater value to the clinician than is generally believed, and that does not require any concessions so far as scientific accuracy is concerned. The clinician is primarily interested in practical considerations. An autopsy report may be excellent for presentation at a pathological society and at the same time of little value and still less stimulus for a gathering of clinicians. We must emphasize this applied scientific attitude. On the other hand, scientific accuracy should not be sacrificed. The autopsy should not limit itself to the establishing of the cause of death. Such an autopsy satisfies only the diagnostic curiosity of the clinician but does not exhaust the value of the procedure and fulfill its purpose. The quality of the autopsy work will have to be emphasized to a greater extent than it was in the past. Poorly conducted autopsies fail to arouse the interest of the clinicians.

Another point of importance, which concerns mainly the editors of scientific journals, is the fact that not infrequently papers and case reports are being published based upon the study of conditions in which a final check-up by means of an autopsy is not included. In some the conclusions drawn are entirely unjustified without an autopsy. The excuse of failing to obtain an autopsy ought not to be accepted on such occasions. That is where editors could exert a great influence.

More attention will have to be paid to the just grievances of the undertakers. There are a few simple rules to be observed from the time that the patient dies until the body is given over to the undertakers which will be of great help to them and will more than anything else help to gain their coöperation. I should like to suggest that the rules to that effect be printed in our journal and be followed by every pathologist performing an autopsy. A great deal of good can be done by speaking to groups of undertakers, by opening schools of embalming in connection with medical schools,

as was accomplished at the University of Minnesota, where such a school has been functioning since 1914.

The discussions on autopsies at our national gatherings do not necessarily penetrate into the rank and file of the profession. It is necessary for every pathologist to inaugurate discussions in his respective hospital on various phases of autopsies. Much propaganda can be carried on in this way.

There is a conspicuous and ever increasing interest in medical subjects displayed in lay journals of various kinds, if judged by the number of popular medical articles printed in such journals. There is an excellent field for pathologists who have the ability to write in a popular manner, to bring to the attention of the reading public the importance of autopsies for public health.

Another phase which still offers great possibilities is an increase of emphasis on the absolute necessity of autopsies in the curricula of our medical schools. They ought to be used to a greater extent than is done now as a basis for instruction in diagnosis, while at the present time autopsies are used mainly for instruction in morbid anatomy.

CONCLUSIONS

The autopsy has been presented as a public health problem, the solution of which should not be left exclusively to the medical profession. It requires the coöperation of public health authorities.

Until the time when such a program is realized it will be the duty of the pathologist to make the medical profession fully conscious of the value of autopsies.

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THE PATHOLOGIST'S DUTY IN OBTAINING PERMISSION FOR AUTOPSY

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The hospital pathologist who concerns himself only with the performance and diagnoses of such autopsies as are requested is shirking a very important part of his duty. Unless he takes an active part and interest in methods of stimulating an increase in the number of autopsies he hinders the scientific advancement of the staff and institution which he serves. The need and value of a larger percentage of necropsies have been well shown by many leaders in the medical profession. Waite¹² stated that "All roads in medicine lead to, or emanate from, the postmortem table." Christian⁵ believed that any hospital service is incomplete without a check-up of the clinical diagnoses in those cases that are fatal and that the autopsy number and percentage is the best single index of the professional efficiency of a hospital. The latter statement is undoubtedly stretching the point a good deal.

A more conservative attitude would be to the effect that the percentage of autopsies is one of the best indices of the scientific attitude of a hospital. Bluestone² was more emphatic; "no hospital is larger than its pathological laboratory. . . . The progressiveness of a hospital is in direct ratio to the laboratory spirit which it maintains." This "laboratory spirit" must essentially emanate from the pathologist, as Bell¹ emphasized, the pathologist must make the physician want to see autopsies on all the clinical cases that die.

Bluestone believed that the medical profession should regard every death from disease as a symbol of their failure to save life. The pathologist therefore, attempts to learn from and help decrease the number of these failures. If the autopsy is regarded as such a valuable adjunct in the study and knowledge of disease

processes and the pathologist is supposedly a leader in this study why, then, should he regard the efforts to increase the number of these studies as a purely administrative and clinical problem and leave that work to others?

The pathologist must incur and maintain the good will and confidence of all members of the hospital staff: internes, house officers, residents, visiting and consulting staff alike. These clinicians are the ones who will, in the end, ask permission for autopsy from the relatives, even beg for it at times. It appears hardly logical to suppose that any human being (and the clinician is no more than human) will force himself to face such situations to supply the necessary work for one whom he dislikes. With an existing feeling of good will the physician will feel a personal interest to obtain this essential examination in every possible case.

The pathologist should encourage the clinical staff to view every patient who is dangerously ill as a possible subject for postmortem examination. Keeping this possibility in mind the attending staff can mold the feelings of the relatives while the patient is still living so that they (the relatives) will feel a return obligation to grant requests that the staff asks of them. There are many excellent papers containing valuable suggestions for use in the development of this "consent psychology" on the part of relatives. It is not the intended scope of this paper to discuss this important phase in gaining permission for autopsies but a partial list of the more important articles is worthy of reference. Bluestone, Bell, Gordon,⁶ Tarr,¹¹ Warwick,¹³ Boyd and Gordon,³ Parkhurst¹⁰ and Charache⁴ have written papers which are recommended to pathologists and clinicians alike for their valuable aids and suggestions. Religious misbeliefs form an important subject in the refusal of permission for autopsy and such objections are cited by Joslin,⁸ Gottlieb⁷ and Lauterbach.⁹

Clinico-pathological conferences are an excellent method of stimulating the staff to want to see autopsies and to verify the clinical diagnosis on their patients that die. The pathologist should urge them. This institution holds such a conference each month. At the meeting, one case is presented as a problem by the staff who treated the patient. The remainder of the staff

discusses it from the psychiatric, medical and therapeutic viewpoints and the pathologist engages in the discussion of the necropsy findings.

The relatives in nearly every instance, wish to share in the knowledge of the findings and diagnoses of the examination. It is much easier to obtain a request for an examination from individuals who know beforehand that they will be told of the causes of death. Without this the relatives consent only on the argument of "assisting science" which is a very impersonal and lofty one. Doctor William A. Bryan, Superintendent of this institution, devised and routinely sends a form letter to the relative of every patient on whom necropsy has been performed. A typical letter of this type is:

My dear Mrs. Doe:

I am writing to give you the results of the examination which you so kindly permitted us to make, following the death of your husband, John Doe.

We find that his death was caused by pneumonia. He had a chronic kidney disease and ulcer of the stomach. There was a marked degree of hardening of the arteries of the brain which accounted in some measure for his mental symptoms.

May I express my personal sympathy to you at this time and assure you that the hospital did everything possible to make your husband comfortable during his last illness. He suffered very little and his death was peaceful. The rites of the Church were administered to him by our Catholic Chaplain.

If the hospital can be of any further service to you at any time, please do not hesitate to call on us.

It is to be noted that this letter is worded in the very simplest of English terms. The relatives receiving such a letter must feel that they too have gained by granting permission. The pathologist would do well to take on such a duty personally, if it is not already done by the institution.

The pathologist must coöperate with the undertakers who receive the bodies on which autopsies have been done. The undertakers compete by making the corpse attractive in the least possible time. It takes two or three extra hours' work to accomplish this even under the best of conditions. When this work is increased because of carelessness on the part of the one who performed the autopsy, is it small wonder that the members

of their profession are antagonistic towards autopsies in general? This ill-feeling can be remedied and has been remedied to some extent in this community.

The pathologist might interview the morticians of his community. Doctors Bryan and Flower have been responsible, in a large measure, for the existing good feeling between the local undertakers and this institution. These doctors interviewed the group and asked for their reasons for previous antagonistic attitudes. To change this attitude it is important that we follow out the suggestions that the undertakers made. By so doing, it must interest them in our getting and doing autopsies. In this connection, Doctor Bryan uses another type of form letter which he sends to every undertaker who collects a corpse on which autopsy has been performed in this institution:

Dear Sir:

This hospital is endeavoring to give the best and most efficient service to the members of your profession. We are anxious to render every assistance to visiting morticians, and in order to check up our methods, I am writing to ask if everything was satisfactory in the case of Jane Doe, delivered to your firm May 17, 1932. An answer to the following questions will be appreciated.

Did you receive prompt and courteous attention when you called for the body?

Was the body in good condition?

Have you any suggestions to offer that will facilitate your work in any way?

Are our autopsied bodies in good condition, and can you suggest any way in which we can improve our autopsy methods?

We desire to coöperate with you in every way, and if anything occurs in the future that requires correction, please inform me at the earliest opportunity.

We have received many interesting and valuable suggestions from these letters. We had one of the group demonstrate his method of embalming an autopsied body in our laboratory so that we could visualize his difficulties and improve our technic to make those difficulties less troublesome. In return we have been more than compensated by the mortician's change of attitude and aid in advising their clients to allow us to perform post-mortem examinations.

The results of these conferences with the undertakers have elicited the following points as essential in every necropsy and

are deemed worthy of elaboration. These routine procedures take very little extra time.

WRIST AND CHIN TIES

The corpse should not have any wrist or chin ties. When the body is "laid-out" on the wards by the nurses it has been their custom to secure the two wrists by tying with a wide bandage. They also close the mouth by bandaging the lower jaw tightly to the head. As rigor mortis sets in, these ties leave visible marks on the corpse at the funeral that are particularly unsightly. Undertakers on the whole prefer that these ties be omitted. The sleeves may be pinned to the body of the shroud to prevent the slipping of the arms. If the chin does sag they much prefer to wire the upper and lower jaws together from the inside of the mouth.

TRUNK

The "Y" incision is used on all trunks, male or female alike. Starting at both acromioclavicular junctions the incisions are extended downward and towards the anterior medial line, meeting at the xyphoid process. The incision is then extended downward in the midline of the abdomen. This method hides any incision marks at the funeral. The wide thorax incision allows the subcutaneous exposure of all the neck tissues (we remove the larynx in every case) and does not distort it even when a low-necked gown is used to dress the corpse. In those cases where the autopsy is limited to the thorax alone the "V" incision is used identically with the upper half of the "Y." In autopsies limited to the abdomen, the inverted "V" incision is used beginning at the xyphoid and extending to the anterior-superior spines of the ilia. This affords an excellent exposure of the abdominal cavity.

All organs are removed and never replaced within the body cavity. Their remnants are burned in the hospital incinerator. If an organ is allowed to remain within the body, once its blood supply is severed which renders embalming of that particular organ impossible, it will putrefy and give off a very offensive odor. Therefore, the undertaker must do what the pathologist carelessly failed to do.

The feces are milked upward in the tract starting from the rectum so that there will be no remaining stools in the colonic stump that is allowed to remain in the body. The stump of the rectal colon is tied at its severed end. The severed end of the vagina is likewise treated after the removal of the uterus.

The remaining stumps of vessels are tied.* The stumps are left sufficiently

* Since writing, the author attended the last convention of the New England Funeral Directors Association. In their unanimous opinion the veins should be left open for free drainage during the process of embalming; only the arteries should be tied.

long (both vessels and twine) so that they may be easily found by the mortician in case he wishes them. The right innominate, left common carotid, and left subclavian arteries are tied in the thorax. The beginning of the two common iliac arteries are likewise treated in the pelvis and the corresponding veins of these vessels are included in the ties. On partial autopsies, if only a portion of the trunk organs are removed, the cut ends of the vessels must be tied.

The empty cavities are sponged and wiped absolutely dry. If this is not done the fluid will soon become odoriferous. The incision of the trunk should be sutured in the same manner as noted above heeding the same precautions.

HEAD

The scalp incision should start just back of the external ear, in the posterior external ear-fold. It is carried on top of the head, well back from the vertex, so that it will not show at the funeral. In case of bald heads the incision is kept within the hairy fringe which is usually present around the occipital portion of the scalp. In this instance the scalp will strip over the face if the ends of the incision are carried downward keeping within the posterior ear-fold. Incisions outside of this ear-fold will show their suture marks at the funeral.

When the brain is being removed the stumps of the two vertebral and internal carotid-middle cerebral arteries are left as long as possible. They are sealed securely by tying with suture material. The corresponding optic nerves may be included in the tie of the internal carotid arteries to give body to the walls of the vessels. The pituitary body may be removed without mutilating the stumps. These ties in themselves are usually sufficient to withstand the force of the embalming fluid except if the vessels are sclerotic and brittle. It is well, therefore, after the cavity is sponged and wiped dry to reinforce the ties by filling the lower cavity with plaster of Paris. It is extremely disagreeable to have bloody-embalming fluid stain the pillow of the casket at a funeral because vessels leak and the fluid seeps through the sutured scalp. Plaster of Paris in itself will not prevent such leaks.†

The upper and lower temporal edges of the cranium must be wired on either side to prevent the upper portion from slipping and showing distortion of the head at the funeral. We use an ordinary small bore, carpenter's bitt and number 18 copper wire.

SPINAL COLUMN

The removal of a portion of the spinal column and cord presents a difficult problem on account of its location as the body lies on its back. Ordinarily this procedure allows fluid to seep through the sutured incision and will stain the

† The funeral directors also disapproved of the use of plaster of paris inasmuch as it may prove to be a nuisance if they should desire to remove it. This procedure would better be left out.

casket lining and clothes, a mishap which is particularly annoying. After the removal of the cord all of the severed vessels are sought and individually tied. The bone fragments are not replaced. The cavity is sponged and wiped dry. The skin incision must be carefully sutured. This technic results in a dry back.

SUTURE AND TIE MATERIAL

A very important point from the mortician's point of view is the suture material. It must not break or it will either allow distortion of the skin of the body or seepage of body fluids and stain the lining of the casket or both. We use Belfast cord which is a starched white cord of very satisfactory strength. The sutures must be close enough to prevent seepage of fluid from the edges of the incisions. We use the baseball stitch, each stitch not more than one-half inch away from its fellow above or below and keeping the string taut results in waterproof-sutured incisions. Particular care must be used to make the incisions absolutely leak-proof in the head and back.

The skin of a body on which autopsy has been done is next washed and wiped clean and dry. At this time the sutured incisions should be closely scrutinized for leaks. If any does occur it can be stopped by close sutures.

The shroud should be clean and dry. No one cares to handle soiled clothing and the undertaker is no exception. At the time the undertaker calls for the body someone conversant with the extent of the autopsy should verbally inform him of it and the vessels that remain tied. If this is not practicable, in those instances in which bodies are called for out of usual hours, a slip of paper containing the necessary information may be left with the body.

The pathologist should never keep the undertaker waiting for a body. At the time the permission is obtained the relatives are asked the name and address of the undertaker who is to call for the body or if they have not as yet decided upon one, they are asked to have the man they select call to make a definite appointment at which time he may receive the body. Once the appointment is made, an hour being added on to the estimated time for completing the autopsy and preparing the body, the body should be ready for delivery before the arrival of the hearse.

The undertaker has just as much right to call for a body at midnight, providing he has the necessary permits, as during the day. Any delay that the institution may cause by opposing such a right will result only in antagonism and defeat the purpose of increasing the percentage of autopsies.

It is justifiable to ask if the results of such an elaborate and extensive procedure has any compensation. One's answer can-

not be more forceful than the actual results. Our present methods in coöperating with the undertakers were started during the early part of 1931. A comparison between the results in the years 1930 and 1932, inclusive, is given in table 1. It is also worthy of note that since the onset of using this procedure up to the present time more than 180 autopsies have been performed with but one case in which it was granted with limitations as to the extent of the examination.

These methods really add very little extra work as far as the pathologist himself is concerned. A well trained assistant can open the calvarium, remove the brain, split the spinal column, tie the cut vessels and suture the skin while the pathologist is remov-

TABLE 1

SUMMARY OF AUTOPSY STATISTICS SHOWING INCREASE IN AUTOPSIES PERFORMED

| | 1930 | 1931 | 1932* |
|-----------------------------------|------|------|-------|
| Number patients died..... | 227 | 220 | 103 |
| Number autopsies performed..... | 75 | 116 | 76 |
| Per cent autopsies performed..... | 33.0 | 52.7 | 70.9 |

* This includes the first five months only.

ing, describing and sectioning the organs. The entire procedure has taken us but two hours in our speediest work from start to finish including the weighing and measuring of every organ.

SUMMARY

Pathologists are urged to take an active part in order to increase the number of postmortem examinations in the institutions which they serve.

Periodic clinico-pathological conferences will stimulate the staff to want to see autopsies performed on the patients who die.

Relatives should, if they so desire, be informed of the results of the postmortem findings. The pathologist must be willing to take on such a duty.

Pathologists must give careful attention to the care of bodies on which autopsies have been performed following the sugges-

tions of the local undertakers. The technic used must be one that aids the embalmer in every detail.

Close coöperation between the pathologist and the undertakers will result in their help rather than antagonism.

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PRIMARY TUMORS OF THE LIVER*

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The main purpose of this paper is to discuss the pathology of primary hepatic tumors and to report eight cases of primary carcinoma of the liver encountered in 1087 necropsies at Detroit Receiving Hospital during the thirty-four months between March 28, 1929, and January 20, 1932. This constitutes an incidence of 0.74 per cent of all autopsies and 8.4 per cent of all carcinomas encountered at autopsy. To this will be added a report of two secondary neoplasms of the liver which possessed features in common with primary tumors of this organ and presented a problem in differential diagnosis. No attempt will be made here to review completely the subject of primary hepatic neoplasms or to collect all of the reported cases. The reader is referred to the articles of Wegelin,²⁷ Castle,⁴ Griffith,¹² Wollstein and Mixsell³⁰ and Dansie,⁷ dealing with primary carcinoma of the liver in children.

The liver may be the site of many rare neoplasms such as cystadenoma, sarcoma, endothelioma, hypernephroma, but those most frequently encountered are of epithelial origin and in the experience of most authors the majority are derived from or resemble liver cells (solid parenchymatous tumors or hepatomas) while the remainder appear to be related to bile duct epithelium (glandular type or cholangioma). Jaffé¹⁶ stated that only forty-eight cases of unquestionable primary sarcoma of the liver had been reported. It is more than possible that many of these primary sarcomas were actually of epithelial origin.

Kelsch and Kiener¹⁸ in 1876 reported two cases of primary carcinoma of the liver and were able to find only one other positive

* Read before the Eleventh Annual Convention of the American Society of Clinical Pathologists, New Orleans, Louisiana, May 6-9, 1932.

case. Sabourin²³ in 1881 added four cases. Hanot and Gilbert¹⁴ in 1888 discussed primary carcinoma of the liver under the separate headings of pathological anatomy, etiology, histogenesis and pathogenesis, symptomatology, diagnosis and treatment including twenty-four in part unpublished observations on this subject. In 1901, Eggel⁸ collected 163 cases and added one of his own; of these 163 cases only 117 had been studied microscopically. Wegelin reported eight cases from the Bern Pathological Institute. Ribbert²¹ added a valuable contribution.

INCIDENCE

While primary carcinoma of the liver is a lesion infrequently encountered it can by no means be considered rare. It is sometimes difficult to judge whether this lesion is extremely rare or relatively common because its incidence appears to vary greatly in different geographical areas. Clawson and Cabot⁵ reported the only case seen in 5100 necropsies at the University of Minnesota. Winternitz²⁹ found three cases in 3700 autopsies at Johns Hopkins Hospital up to 1916 which constituted an incidence of 0.028 per cent of all postmortem examinations. Heller (quoted by Ascoli²) found primary hepatic tumors in only 0.02 per cent of all patients at the Leipzig Clinic and all of these were diagnosed sarcoma. In 1924, Fried¹⁰ reported five of these tumors, one of which was the only case which had been found at autopsy at the Harvard Medical School up to that time.

The other extreme is found in the written reports of other observers. Smith²⁴ reported twelve cases of primary carcinoma of the liver (nine hepatomas and three cholangiomas) collected during one year in the Philippine Islands in 872 consecutive autopsies, an incidence of 1.4 per cent, or 17 per cent of all cases of malignancy found at autopsy during that time. Strong and Pitts²⁵ collected nine cases in eight years (1920 to 1927) at the Vancouver General Hospital in 1024 autopsies (0.87 per cent). Eight of these nine patients were Chinese, making the autopsy incidence 0.11 per cent in whites and 6.9 per cent in Chinese. Pirie²⁰ at the South African Institute for Medical Research, in the nine years from 1912 on, found that in the routine examination

of 3900 tissue specimens a diagnosis of primary carcinoma of the liver had been made in fifty-two cases, thirty-six of which appeared to him authentic; and that it was the commonest tumor in African natives, a race in which malignant disease is exceptionally rare.

An average incidence is recorded in the reports of others such as Allbutt and Rolleston¹ who found twenty-four cases in 18,500 autopsies (0.12 per cent); Goldzieher and Bokay,¹² eighteen in 6000 autopsies (.03 per cent); Rowen and Mallory,²² nine in 6506 (0.13 per cent); Wheeler,²³ 15 in 5233 (0.28 per cent); Counseller and McIndoe,⁶ eight in 5796 (0.14 per cent) and von Glahn and Lamb,¹¹ six in 1800 cases (0.33 per cent). The average of all reports would be about 0.2 per cent.

CASE REPORTS

*Six hepatomas**

Case 1. A white male, sixty-five years of age, was admitted March 23, 1929 complaining of colicky pain in the upper right quadrant radiating to right shoulder for nine months and abdominal distention for three months associated with intensive jaundice and a twenty-five pound loss of weight. He had drunk two quarts of beer daily and whisky occasionally for fifty years. Hemoglobin was 24 per cent. Kahn and Kolmer tests were negative. Death occurred on March 28, 1929. Necropsy showed that the liver weighed 2100 grams and contained numerous new growth areas which in some portions had coalesced to form larger multinodular masses. The surface was nodular; the liver parenchyma was very hard and offered resistance to cutting. This is the only case in this series in which cirrhosis was present (figs. 1 to 4). The diagnosis was hepatoma with cirrhosis.

Case 2. A white male, aged fifty-two years, entered October 9, 1930 complaining of abdominal pain without jaundice. There was a typical history

* Since this paper was read the author has encountered at autopsy three additional cases of hepatoma, one of which was associated with portal cirrhosis. One of the remaining two was an incidental finding, represented a very early stage, and consisted of a diffuse distribution of many minute nodules, supporting, it would seem, the multicentric theory of origin.

These cases increase the autopsy incidence to 0.85 per cent, change the ratio of hepatomas to cholangiomas to nine to two and revise the incidence of cirrhosis associated with hepatoma to two in nine cases and with all cases of primary carcinoma of the liver in this series to two in eleven.



FIG. 1. TYPICAL SPECIMEN OF HEPATOMA WITH CIRRHOSIS



FIG. 2 APPARENT TRANSITION FROM NORMAL LIVER CELLS TO NEW GROWTH TISSUE

Note preservation of typical liver vascularity in carcinoma tissue



FIG. 3. SMALL NEOPLASTIC AREAS. SOME INTRAVASCULAR, IN AN AREA OF CIRRHOSIS

This picture suggests that these areas of carcinoma were in the beginning simple nodular hyperplasia.

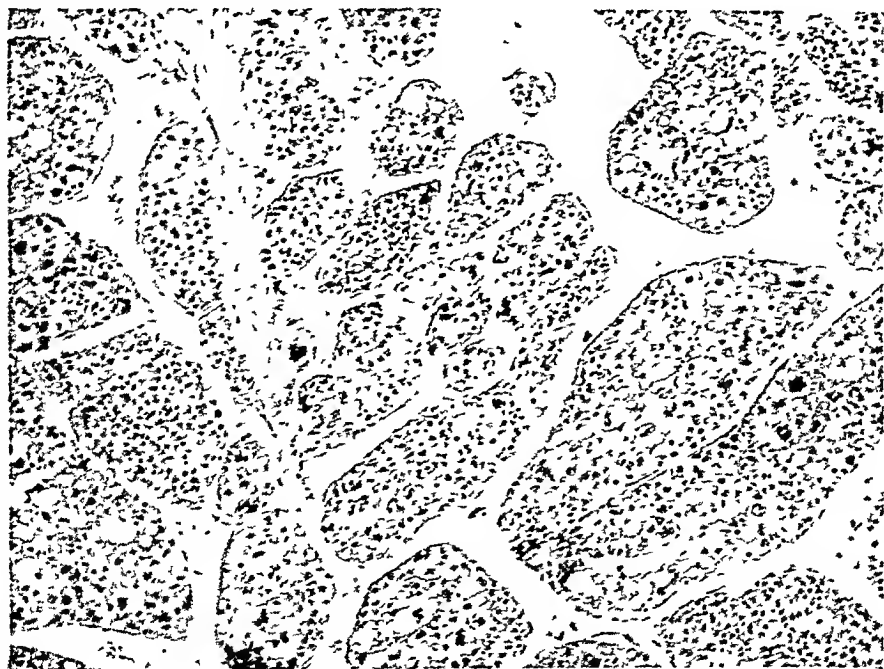


FIG. 4. PAPILLARY AND FATTY DEGENERATION IN HEPATOMA

This tissue is undergoing the same type of degeneration commonly observed in liver parenchyma.

of peptic ulcer or gall bladder disease for the past six years. Laparotomy was performed. Death occurred five days later on October 22, 1930. Neeropsy showed that the liver weighed 3800 grams and was diffusely mottled throughout by white and yellow nodules varying in size from 1 to 3 mm. There was metastasis to both lungs, to peribronchial, anterior mediastinal and retroperitoneal lymph nodes and to the pancreas. The diagnosis was multiple hepatoma metastasizing to lungs, mediastinal and regional lymph nodes and pancreas.

Case 3. A forty-six year old white male was admitted on July 27, 1931 complaining of a mass in abdomen, dyspnoea, weakness, jaundice, loss of weight and vomiting for six months. The patient was severely anemic and gave a definite history of alcoholism. Icterus index was 15. Death occurred August 15, 1931. At neeropsy the abdomen contained bloody fluid. The liver weighed 5300 grams and contained numerous tumor masses throughout. There was also a large mass of new growth tissue, 15 cm. in diameter, attached to the spleen and producing pressure on the diaphragm and on the cardiac end of the stomach. Thoracic and abdominal cavities were otherwise normal. The diagnosis was multiple hematoma metastasizing to the spleen.

Case 4. A white female, aged sixty-three, was admitted on November 2, 1931 complaining of vaginal hemorrhage. She insisted that she had had no symptoms previous to this acute attack. The abdominal wall was thin. The liver extended two finger breadths below the costal margin and contained a definite palpable hard mass above and to either side of the umbilicus. The patient expired November 3, 1931. Neeropsy showed that the vaginal hemorrhage had been caused by a submucous myoma of the uterus. The liver was enlarged and contained numerous masses, the largest of which was 2 cm. in diameter. Metastasis had occurred only to the regional lymph nodes. Diagnosis was multiple hepatoma with metastasis to regional lymph nodes.

Case 5. A white male, aged seventy, came to the hospital because of painful enlargement of the abdomen, the presence of a mass in the upper abdomen, a thirty-pound loss of weight, vomiting and weakness. He was well until six weeks prior to admission. A mass about the size of a grapefruit, apparently continuous with the liver, could be palpated. The mass was extremely painful. Icterus index was 10. Blood count showed 120,000 leukocytes with 83 per cent polymorphonuclears. Death occurred December 14, 1931. Neeropsy showed that in the medial portion of the right lobe there was a spherical mass 17 cm. in diameter, bulging the capsule anteriorly and extensively invading the overlying omentum. The central portion of the mass was extensively necrotic. Diagnosis was solitary hepatoma. There were no metastases.

Case 6. A sixty-two year old white male, entered November 6, 1931. Three weeks after recovery from an attack of pneumonia he became jaundiced. The jaundice deepened. There was ascites. The liver was smooth to palpation and extended two finger breadths below the costal margin. He had a moderately severe secondary anemia. Icterus index was 40. He had been a saloon keeper before prohibition. Death occurred January 20, 1932. At necropsy a large amount of clear fluid was found in the abdominal cavity. There were innumerable small new growth nodules throughout the liver with metastasis to the coeliac nodes and the capsule and lymphatics of the pancreas. Diagnosis was multiple hepatoma with metastasis to regional nodes and pancreas.

Two cholangiomas

Case 1. A colored male, forty-four years of age, was admitted April 14, 1929. There had been swelling of lower extremities, dyspnoea, pain in back and cough for one year. Spinal fluid and blood Wassermann were negative. Death occurred June 6, 1929. At necropsy the liver weighed 5800 grams. Throughout both lobes there were tumor masses, the largest of which measured 15 cm. in diameter. The larger masses were fluctuant. The only evidence of metastasis was a nodule 1 cm. in diameter in the wall of the right auricle slightly above the tricuspid valve. (For microscopical picture see Fig. 5.) The diagnosis was cholangioma metastasizing to the heart.

Case 2. A white male, sixty-two years of age, entered the hospital on March 21, 1931, with a diagnosis of chronic alcoholism. He appeared anemic but not jaundiced. The liver in the midline extended to within two finger breadths of the umbilicus and then curved downward almost to the iliac crest. He was semicomatose and expired on March 24, 1931. At necropsy the liver weighed 3000 grams and contained numerous fairly large tumor masses, the maximum diameter of which was 3.5 cm., and also a finer, smaller and more diffuse new growth infiltration. The microscopical picture was similar to Fig. 5. The diagnosis was cholangioma without metastases.

Two melanomas

Case 1. A white male, fifty-two years of age, was admitted November 16, 1927. The symptoms were referable to the urinary tract. He had had a mole removed from his back one year before. Death occurred November 29, 1927. Necropsy showed that death was apparently due to urinary obstruction produced by prostatic hypertrophy. The liver was slightly enlarged and contained numerous pigmented new growth nodules. Microscopically the cells were spherical, polyhedral and spindle-shaped. The intracellular pigmentation was light. The liver was the only organ involved. The diagnosis was melanoma of the liver probably primary in previously excised mole.

Case 2. A fifty-five year old white male entered September 15, 1930, complaining of severe abdominal pain, distention, and constipation. There was no history of nausea, vomiting or jaundice. It was thought that his abdomen contained fluid. Icterus index was 15. Three years before his left eye had been injured and became sightless. Death occurred September 29, 1930. At necropsy the liver weighed 9000 grams, was almost black in color and was diffusely speckled with white nodules from 2 to 15 mm. in diameter. No other organs were involved except the left eye which was atrophic and collapsed and



FIG. 5. TYPICAL CHOLANGIOMA

Metastasis in this case consisted of a solitary nodule in the right auricle of the heart.

contained a melanoma of the choroid 5 mm. in diameter. The cells were nearly all spindle-shaped and heavily pigmented. Diagnosis; Melanoma of the choroid with extensive metastasis to the liver.

DISCUSSION

Primary hepatic tumors have always held the interest of the pathologist because of their rareness and because of the many controversial points which the subject precipitates. Discussion in the past has dealt with the following phases: (1) the com-

parative rareness of the lesion; (2) the source of the new growth cells and the incidence of two principal microscopic types; (3) the relationship of adenomas and carcinomas; (4) the gross classification of these tumors; (5) the unicentric or multicentric origin and whether there is transition from normal liver cells to new growth cells; (6) the frequency of cirrhosis as an associated lesion and its rôle as an etiological factor; (7) the part played by chronic hepatic disease such as parasitic infections and chemical damage in the origin of primary malignancy; (8) the comparative infrequency of metastasis.

It is quite generally agreed that hepatoma is more common than cholangioma. Eggel found that ninety-three out of 106 cases were of the liver-cell type. Yamagiwa's³¹ ratio was twenty-seven to fifteen; Rowen and Mallory found seven hepatomas out of nine cases; Winternitz and Muir's¹⁹ cases were all of the liver-cell type. Goldzieher and Bokay found fourteen hepatomas and seven cholangiomas. Nine of Smith's twelve cases were hepatomas. Strong and Pitts found only three cholangiomas in nine cases. In Pirie's thirty-six cases, twenty-eight were diagnosed hepatoma, three cholangiomas and five mixed tumor. Ascoli, in a recent article, had collected in eight and one-half years sixteen cases in his own material of which nine were microscopically malignant (autopsy and biopsy at operation). Of these nine cases two were diagnosed cylinder cell epithelioma, one solid carcinoma, two fusocellular sarcoma, two cholangiocellular sarcoma, one cancer cirrhosis, and one primary central sarcoma composed of round and spindle cells. The case of central sarcoma consisted of a solitary tumor the size of an adult's head. In two cases there were massive tumors with smaller nodules scattered throughout the liver. Advanced cirrhosis was associated with one case and mild cirrhosis with another. In spite of the diagnoses given, six of the cases were probably hepatomas and two were cholangiomas. The same author also reported a primary melanoma of the liver. Exceptions to the predominance of the liver-cell group were reported by Fried who made a diagnosis of cholangioma in four of his five cases, and Karsner¹⁷ reported nine cases, five of which were cholangiomas. The doubt concerning

the relationship of bile duct and hepatic cells, the possibility of transition from one to the other, and the apparent common ancestry of these cells might lead one to think that the classification of these carcinomas was a matter of minor importance and perhaps over-discussed. The clinical and pathological similarity of these two tumors might argue for a single diagnosis for the two epithelial types, and there can be no great objection to calling them all hepatomas.

The division of epithelial new growths of the liver into adenomas and carcinomas might be frowned upon on the basis that all adenomas are either actually or potentially malignant and therefore should be diagnosed and treated as carcinomas, although it is recognized that benign adenomas have been successfully enucleated.

A gross classification of these tumors is probably unimportant, inasmuch as the fundamental type in all might be the same, the physical characteristics only being different. Ewing's⁹ nomenclature (dividing hepatomas into (1) solitary adenomas or hepatomas, (2) massive and (3) multiple carcinomas or hepatomas and (4) carcinomatous cirrhosis), is as useful as any and furnishes us with gross descriptive terms.

The controversy regarding the unicentric or multicentric origin of these tumors will probably not be settled for some time. Karsner, Winternitz, Ribbert and others have favored the unicentric origin. Van Heukelom,¹⁵ Travis,²⁶ Counseller and McIndoe and others believed that they demonstrated that the origin may be multicentric. It is argued by the latter that in their case dissemination could never have been explained by thrombosis and embolism of the portal or hepatic vessels unless the emboli traveled against the blood or lymph flow which they term improbable. However, in one of my cases there was a large secondary mass in the spleen which makes one think of retrograde extension through the portal and splenic veins. This point, however, has very little practical importance.

The frequency of cirrhosis as an associated lesion and its rôle as an etiological factor is of considerable importance. It is the general impression by nearly everyone who has written on this

there was a fairly characteristic architecture of these tumors with cord arrangement of cells and a vascularity which simulated or imitated normal sinusoids. Differentiation into bile ducts should probably be looked upon with suspicion. In many of these lesions differentiation is sufficiently complete to produce cells and a cell pattern so closely resembling normal hepatic parenchyma as to be quite unmistakable. Such a picture obtains in the so-called adenomas. In other instances differentiation is apparently poor and atypical patterns are produced; in such cases a positive diagnosis of primary carcinoma of the liver could hardly be made without a complete autopsy.

Two cases of metastatic melanoma of the liver are described both of which might have been considered primary in the liver and are no doubt as much primary as most of those previously reported. In one case there was an old injury to one eye to which no clinical suspicion of malignancy was attached. This eye was removed and sectioned more or less as an afterthought and might easily have been omitted in a routine autopsy and the primary site left undiscovered. In the other case no primary lesion was demonstrable but the history of the removal of a mole one year before is probably significant.

While metastasis to any organ is possible, the new growth process may remain confined to the liver and when extrahepatic involvement occurs, the lungs and the mediastinal and upper abdominal lymph nodes are the principle sites of secondary deposits. Of the latter there are several possibilities. In the six hepatomas here considered, there was metastasis to the upper abdominal lymph nodes in four instances, lungs and pancreas each twice, and the spleen and mediastinal nodes each once. Of the two cholangiomas there was no metastasis in one case and a secondary lesion only in the right auricle in the other. This experience, however, does not follow Ewing's statement that "extrahepatic metastases are much earlier and more frequent in biliary cancer than with hepatoma." In two of the cases in this series the capsule of the pancreas was quite extensively involved and the lymphatics of the pancreas were distended by new growth

masses. It is to be remembered in this connection that considerable lymphatic drainage from both the liver and pancreas goes to the coeliac nodes and from here retrograde dissemination of a hepatoma to the pancreas could occur. The peritoneum of the upper abdomen is frequently involved probably due to direct extension from involved lymph nodes.

CONCLUSIONS

(1) Eight cases of primary carcinoma of the liver were encountered in 1087 consecutive autopsies representing an incidence of 0.74 per cent.

(2) Six of these tumors were hepatomas and two cholangiomas.

(3) The patients were the type usually encountered in a charity hospital in any large city. Alcoholism and syphilis appear to play a minor etiological rôle.

(4) Cirrhosis was associated with only one of these cases (a hepatoma), yet no attempt is here made to dispute the reported relationship between cirrhosis and primary carcinoma of the liver in the experience of others.

(5) The two cases of secondary melanoma of the liver illustrate that probably most primary melanomas of the liver which have been reported are only apparently primary there.

(6) Diagnoses of primary sarcoma of the liver should probably be viewed with suspicion on the ground that many so diagnosed are of epithelial origin.

(7) A much higher percentage of primary carcinoma of the liver is apparently found in autopsies on patients in whom chronic hepatic diseases are prevalent.

(8) The gross appearance of hepatomas is not especially helpful in identifying the lesion but the histopathological picture is fairly distinctive and positive microscopical diagnosis can usually be made.

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DISCUSSION

DR. I. DAVIDSOHN: The striking feature of Dr. Brines' report is the infrequency of cirrhosis in his cases of primary carcinoma of the liver which is as he himself emphasized, in such a sharp contrast to the prevalent opinions in the literature. In this connection it may be of interest that the question has not been settled as to whether the cirrhosis of the liver precedes or follows the development of the malignant process.

OBSERVATIONS MADE DURING A STUDY OF THE CYTOLOGY OF ENDOMETRIUM*

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During a study of the cytology of the endometrium in more than 100 fresh specimens obtained at operation, together with an equal number of specimens obtained from the pathologic museum, certain observations were made. Some of these are summarized in this paper.

The appearance of fresh tissues prepared by the freezing method and examined immediately was definitely different from that of the tissue in fixed sections. The shrinkage in fixed specimens was roughly 10 per cent of the nuclear diameter. The shrinkage from evaporation, exudation, and contraction of the tissue elements in specimens removed at operation was marked during the first hour after their removal. This shrinkage necessarily led to considerable distortion and to other artefacts. It was apparent that cells varied considerably in size and shape depending on their situation and that they moved far more than has generally been thought.

The mucosa appeared to vary between 1 and 5 mm. in diameter and there was no distinct division into layers. Each millimeter of added thickness corresponded roughly with one postmenstrual week. The stroma (fig. 1) consisted of a fine, loose network including blood vessels, lymph tracts, reticulo-endothelial cells, lymphocytes, and an occasional lymph follicle. The cervix differed in that the glands were of the racemose type and definitely

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FIG. 1



FIG. 2



FIG. 3



FIG. 4

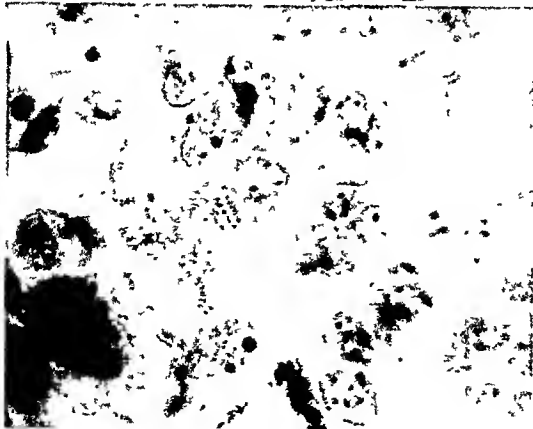


FIG. 5

FIG. 1. STROMA CELLS ($\times 600$)

FIG. 2. CILIA ON ENDOMETRIUM ($\times 600$)

FIG. 3. SECONDARY CYTOPLASIA ($\times 600$)

FIG. 4. REGENERATION CELLS ($\times 600$)

FIG. 5. MALIGNANT CELLS ($\times 600$)

mucus-forming, with the stroma containing much more fibrous connective tissue. The cells of the endometrium varied with age, menstrual phase, pregnancy, ovarian changes, luteal reactions, infections, the presence or absence of neoplasms and probably in the presence of abnormal conditions of the thyroid, pituitary, suprarenal, or mammary glands. There was a continual change going on and regeneration following menstruation was very rapid.

Fetal endometrium¹ which forms at about the twelfth intra-uterine week, presented a rather homogeneous structure. The stroma was densely filled with cells resembling early fibroblasts or histocytes. These might be referred to as histoblasts.

In premenstrual endometrium there was underdevelopment of the whole structure, although this was less marked in the cervix. Cilia (fig. 2) were seen in twelve of the fresh specimens, without any particular search, and in these cases there appeared to be, from the history, some association with infection.

Adult endometrium was fully developed. There was marked variation in individuals, depending principally on the menstrual cycle, unless neoplasm was present. The cells concerned were chiefly the histoblasts present in the stroma, which, as elsewhere in the body, form the chief elements of repair. They are early embryonic types of pleomorphic cells. They appear to be closely related to the monocytes. These cells were fully studied by Maximow.² I have observed them undergoing transformation from connective tissue elements into smooth muscle cells in the uterus of the rabbit. They are present in all connective tissue throughout the body; for instance, that in the spleen, bone marrow, sinusoids of the liver, lymph nodes and mucosa and surrounding smaller blood vessels; moreover, they are found in the omentum,⁴ where they form, becoming more or less fixed lymph channels which, under abnormal conditions, develop into lymphatic channels of adult endothelium. From this cell, I believe, develop the several varieties of giant cells, the epithelioid cells, the Kupffer cells, the dust cells, the heart failure cells, the cholesterol macrophages, the decidual cells, the elements of the corpus luteum, the fat cells, and the pigment cells. These cells, apparently, are most important in immunity reactions and in infections.

That they are the cells concerned in malignancy has yet to be proved but it seems that they constitute the "repair" cells (regeneration cells), which are seen in neoplasms in the indefinite zone between the regions of benignancy and malignancy. One cannot help but feel that these cells appear to take on malignant characteristics.

A peculiar structure was encountered, which, under low magnification, might be taken for a lymph follicle, but under higher magnification there appeared a concentric arrangement of two types of stroma cells, greatly hypertrophied. There were changes in the surrounding stroma and glands. Another similar structure was seen adjacent, and in several instances the cells were undergoing change on one side of a gland similar to that seen at the border of malignant growths in the stomach and colon. The specimen was from a nullipara, fifty-one years of age. The history was that until eight months before operation menstruation always had been regular. Then menorrhagia and metorrhagia had appeared. There was a history of malignancy in the family. The pathologic diagnosis was multiple fibromyoma, hypertrophic endometrium, and chronic cystic cervicitis. The possibility of a decidual reaction had to be considered, but it was remarkable that a similar structure was present in several other cases in which the diagnosis was carcinoma, and in which two types of similar cells could be seen replacing glands. In one case this formation was present on the surface.

Postmenstrual endometrium presented evidence of diffuse abiotrophy with more or less marked sclerosis.

Pregnancy normally caused diffuse hypertrophy and hyperplasia of the histoblasts, forming decidual cells. There were specimens from the abnormal condition, hydatidiform mole, with its occasionally resulting malignant conditions, chorio-epithelioma.

Infection in the endometrium is particularly well guarded against, especially in the cervix, and judging from the results of some experiments done on animals, it would appear that there is a quick cytologic response to infection, with active outpouring of lymph and of phagocytic cells. Moreover, a wonderful internal barrier of defense cells is soon set up. Menstruation throws out much infection, which usually is superficial.

There seemed to be a definite relationship between neoplasms and hypertrophy and hyperplasia. This was evident in polyps, adenomas and carcinomas, and in fibromas, myomas and sarcomas. It was also evident in the most malignant types, in which carcinoma and sarcoma were indistinguishable. That we have been overwhelmed by classifications is evident when, to describe certain arrangements of tissue, the term "sarcomatous-carcinoma" is used without hesitation. The common condition "fibro-adenoma" is well known.

All the usual types of malignancy were seen in the endometrium. MacCarty's⁵ primary, secondary and tertiary cytoplasia were demonstrated, and Broders' system of grading² could be applied as well here as elsewhere.

There was no sharp dividing line between a benign and a malignant condition. The so-called regenerative cells (figs. 3 and 4) were potentially malignant (fig. 5), and were pleomorphic.

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CAPILLARY WEAKNESS IN BACTERIAL ASTHMA*

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This discussion is concerned with twenty-three patients with asthma in whom a specific allergin was not demonstrated by the scratch tests, nor did the history and trial elimination give any clue as to a specific offender. Of 1074 patients with asthma classified by Rackemann²⁹ 61 per cent belonged to the groups designated "intrinsic" and "unclassified" showing the prevalence of these types while only 39 per cent was "extrinsic" in nature. I would regard my twenty-three patients as "intrinsic" asthmatics and further would divide the cases into the following subgroups: four, bacterial asthma of children; thirteen, bacterial asthma of adults, and six, reflex asthma, nose and throat only.

ONSET

Because of the confusion which results from the various explanations of this symptom complex, I constantly keep before my mind that the condition represents an impairment of health, a weakened physical condition. Each child began to have his asthma before the age of two years and without having had any recognized infectious disease, although in each there was the history of coryza preceding every spell of asthma. In three of the patients one parent or grandparent had suffered from the disorder. Cooke, Flood and Coca⁶ showed that inheritance exerts a definite effect on the age at which symptoms appear and Kahn¹⁹ believed that there is a hereditary factor in all patients with asthma. One can inherit abnormal tissue reactions and suffer asthma as a result even with a good constitution. On the other hand, one may begin life poorly constructed because of a toxic

* Read before the Eleventh Annual Convention of the American Society of Clinical Pathologists, New Orleans, Louisiana, May 6-9, 1932.

mother who supplied poor material for development in utero. This was demonstrated in a child who had rickets at birth, who had a pigeon breast, and whose lower ribs were depressed reducing the capacity and flexibility of the chest to a great extent.

The most impressive fact of the adult bacterial group is that in eleven of the thirteen, the onset began during, or shortly after, a severe infectious disease, pneumonia in seven, and influenza, of 1918 or 1919, in four. In another patient the onset occurred after the birth of three children within three years, but in the remaining patient there was not a definite association with any physical strain. The average age at onset was twenty-five years, the youngest was twelve and the oldest forty-eight. There was a family history of asthma in only one, a man whose asthma began after pneumonia at the age of thirty-four. The patients of this group originally had good constitutions but they had been injured by two or three infectious diseases as measles with or without serious pulmonary involvement, scarlet fever, diphtheria, whooping cough, rheumatism; probably the usual percentage had primary tuberculous infections and then came another severe strain such as pneumonia, influenza, or repeated pregnancies.

Patients in the group having nasal polypi began to have their symptoms at a later age, the average for six patients was fifty-one years but the onset was not closely associated with any particular disorder. However, these patients have had the diseases of childhood and numerous other infectious ailments. Throughout life they have persecuted their bodies by loss of sleep, by eating poor food or too much food, by excessive use of tobacco and alcoholic drinks, by overwork or too little work, by anxiety, by worry, by trouble, and by the stress and strain of living. With these habits of dissipation are often associated the focal infections, or perhaps the latter are the results of the former.

ALLERGY

There is some change in the tissue reactions of an asthmatic patient which makes his respiratory organs respond to various foreign substances and stimuli; there is sufficient experimental evidence to show that both the body cells and the blood partici-

pate in these reactions although the explanation is theoretical and the actual state of affairs has not been demonstrated. The reactions in the lungs which are of chief concern are the contraction of bronchial muscle and the inflammatory changes of the mucosa with congestion, vasomotor weakness with extreme relaxation of the vessels followed by extravasation of serum and cells, especially eosinophils, into the tissue, and there is still a debate as to which is more important. Manwaring²⁵ believed that increased specific capillary permeability will be shown to be the dominant fundamental physiologic change in protein sensitization to which all other anaphylactic reactions are secondary. Work on man and animals has demonstrated the highly specific nature of the allergic reaction but the inflammatory state of the respiratory tissue in asthma can be produced by so many factors that the specific allergic reaction does not explain the whole picture. The inflammatory state responsible for at least a portion of the asthmatic syndrome may be the residue of an acute respiratory infection.

THE MUCOSA OF THE RESPIRATORY TRACT IN ASTHMA

I have never examined the nose of an asthmatic patient during the symptomatic stage without finding it boggy, swollen, and wet. Nasal polypi represent collections of fluid with slight, fibrous stroma infiltrated with eosinophils, lymphocytes, and plasma cells; the same microscopic appearance is seen in the thickened scrapings of sinuses and in polypoid nasal mucosa. Portions of nasal epithelium obtained at autopsy by Kountz and Alexander²¹ showed prominent glands undergoing mucoid degenerations, hyperplasia of the epithelium, edema, congestion, and thickening of the submucosal layer with cellular infiltration. In cases examined bronchoscopically during paroxysms, Clerf⁵ found marked swelling and turgescence of the mucosa which appeared cyanotic and purple rather than red. In practically every case there was an increase in the quantity of secretion; this varied considerably. With suppuration the secretion was purulent, without odor, and could be traced to definite branches usually those of the lower lobes. In a larger group of cases, the secretion

mucoid in character, was found adherent to the mucosa of the trachea and bronchi. It often practically occluded the orifices of the smaller bronchial subdivisions. Owing to the tenacious character, great difficulty was experienced in its removal either with swabs or by a specimen collector. Objective evidence of spasm of the bronchi or trachea was not found in the cases examined. Jackson¹⁸ found pulmonary secretions to cause partial or complete block of smaller bronchi by their valve-like action, and in many cases of asthma the air hunger seemed to be due to pumping out of air from both lungs. Bronchoscopic removal of the secretions that were acting as check valves almost always cured the air hunger. Autopsy reports are few but the findings as described by Rackemann,²⁸ Huber and Koessler,¹⁷ Kountz and Alexander, and Bergstrand³ were fairly uniform. The epithelial and sub-epithelial layers of the bronchi were essentially similar to those of the nasal mucous membranes. The goblet cells and bronchial glands seemed to have a conspicuous part in the process. There was intense congestion, some edema and cellular infiltration, the muscle layer of the 3 to 6 mm. bronchi was thickened while the walls were thinner and relatively more dilated in the 2 to 3 mm. bronchi; this latter finding gave support to the theory of muscle spasm as a cause for asthma. The lumen contained exudate of serum, mucus, and cells of varying degree, some of the small bronchi were so filled with exudate that the lumen was occluded completely. The parenchyma of the lung showed congestion and edema, emphysema, cellular infiltration, especially eosinophils, and some alveoli were filled with coagulated secretion.

RELATION OF ENDOCRINE GLANDS

Injections of adrenalin have always given remarkable relief in paroxysms of asthma and the relief is proportional to the degree of shrinkage of the mucosa, apparently restoring the vascular tone. I am not prepared to say how extensive is this shrinkage or whether it extends over the entire respiratory tract. I injected lipiodol into the maxillary sinus of one patient and found marked thickening of the mucosa. The patient was given 0.6 cc. of

adrenalin subcutaneously; twenty minutes later another roentgenogram was made but there was no change in the thickness of the mucosa. However, I do know that such shrinkage occurs in the mucosa of the nose and throat. The action of adrenalin is sympatho-mimetic and there is some reason to believe that the injection of adrenalin may stimulate the suprarenal glands to secretion through its acting on the sympathetic terminations in the glands, but this has not been conclusively established. I have personally experienced the effects of an intravenous injection of 0.5 cc. of adrenalin; the significant reaction is a painful, throbbing sensation over each kidney and in the head, with an expansile sensation in the head, this is accompanied by intense nervousness and rapid forceful heart action.

Vascular tone has been associated with the secretions of the adrenal medulla and the posterior lobe of the pituitary, but there are no data to appraise the importance of adrenalin and vasopressin in maintaining arterial tone. Although evidence was far from complete, Dale⁹ thought a really important function of these harmones in relation to the normal circulation may be found in their power to check and to restrict within physiological limits the depressor effects of substances dilating the small vessels when they escape into the circulation. Probably the most acceptable function of the adrenal medulla constitutes Cannon's "emergency theory" whereby it is fitted for temporary reinforcement, during emergencies, of the effects of true sympathetic nerves on almost all organs supplied by them, especially on the circulation. Kellaway and Cowell²⁰ reported prompt acceleration of adrenalin output in the cat when a small dose of histamine was injected intravenously; the effects were slight and evanescent due to the balanced antagonism as compared with the grave and prolonged depressor effects of the same small dose of histamine in a cat deprived of the suprarenal medulla. This functional antagonism of adrenalin and histamine may give another and more direct hint to their functional importance. Hoskins¹⁶ concluded that the collapse of the circulatory mechanism following adrenalectomy is proof of a definite functional interrelationship and Coomb's⁷ experiments led her to believe that some product of the

adrenal tissue, medullary or cortical, is necessary for maintaining normal sympathetic nerve activities on the blood vessels.

The pituitary vasomotor principle, vasopressin, has a more prolonged action upon the vessels when injected, increasing the tone of the minute vessels without any discrimination corresponding to their nerve supply. Krogh²² working with frogs presented evidence that led him to the conclusion that the pituitary vasoconstrictor has the function of maintaining the normal tone of the capillaries and therewith the normal restricted permeability of their walls. On the other hand there is no evidence that a recognizable loss of capillary tone follows removal of the posterior pituitary lobe in mammals. Dale suggests that vasopressin is another safeguard against circulatory collapse by a general and indiscriminating maintenance of tone in the small blood vessels when sympathetic impulses and adrenalin alike begin to fail under the stress of a prolonged and excessive demand for their action. He also offered very strongly suggestive evidence to show that adrenalin and vasopressin can restore tone after its loss by destruction of the nerve supply.

The medullo-adrenal secretion is taking second place in importance to that of the cortex since recent work has shown the really vital function of the cortex. Some uncertain observations associated the adrenal cortex with respiratory movements. It has been confirmed experimentally by Menten and Crile²⁷ and by Menten²⁶ who found that adrenalin endowed venous blood with the power to become converted into arterial blood and enable hemoglobin to become changed to oxyhemoglobin. Various symptoms observed in bilateral adrenalectomized animals are analagous to conditions often encountered in asthma. These animals very early show decreased arterial pressure, metabolism is disturbed, anorexia and gastro-intestinal disturbances occur and are followed by asthenia. The total work done by adrenalectomized rats before complete fatigue sets in was shown to be only one-sixteenth of that carried out by control animals. Many authors have shown that adrenalectomized animals are particularly sensitive to cold and various toxins, as morphine, nicotine, acetonitrile, histamine, and diphtheritic toxin. The effects on

the gastro-intestinal system were severe; Stewart and Rogoff²¹ emphasized the frequent occurrence of gastric ulcer, Alvarez¹ and associates found marked functional derangements. Banting and Gairns² demonstrated extensive pancreatic degeneration of longer lived dogs. Britton⁴ found the pancreas of adrenalectomized marmots which lasted throughout the winter and perished in the spring to show extreme disturbance; usually the gland was very shrunken, thin and congested in appearance; on section it showed hemorrhagic patches, diffuse sclerosis and atrophy. Since severe disturbances of the digestive system is evident following adrenal removal, it is fair to assume that similar conditions are present in many toxic and infectious diseases. It is entirely possible that some of the digestive disorders in asthma have such an adrenal basis. Rowe²⁰ stated that skin reactions are negative in about 50 per cent of patients with food allergy. The deleterious effects of infectious disease on the adrenals was clearly shown by Elliott in autopsy specimens. According to Crowden⁸ the application of external cold produces exhaustion of the adrenalin store of the suprarenal glands of cats provided a marked fall in body temperature occurs and persists. The vital function of the adrenal suffers with the general health as a result of the evil influences of bad habits of living and infectious disease. Think of the severe shock to the adrenal of a boy when he becomes sick from his first smoke or chew of tobacco and assign a proportional blow from each similar offense when the habit continues over a period of years. The effects of poor food were shown by Weil³² in the statement that pellagra, a disease of persons obtaining much of their protein from maize of which the chief protein, zein, lacks tryptophan and lysin, greatly resembles Addison's disease. Also an increase of the cortex at the expense of the medullary portion of the adrenal was found in pigeons with an experimental avitaminosis produced by feeding them exclusively with polished rice.

OTHER FACTORS IN RESPIRATORY INFECTIONS

The specific allergic reaction has not been so definitely established in cases of bacterial asthma and the dyspnea often results

from the local irritation due to non-specific chemical or physical irritants introduced in large dosage. Other factors may produce an inflammatory state of the mucosa, making it more sensitive; thereby responding to non-specific irritants introduced in smaller dosage; and dyspnea may result from the mucosal swelling alone.

It is widely recognized that existing asthmatic states are made worse, or fresh attacks frequently precipitated, by fatigue. Holman¹⁵ believed that allergy in the cell is in many ways analogous to the familiar fatigue phenomenon in which an individual at a certain stage of tiredness is hyperactive, highly irritable and often does more harm than good to himself and his associates. The fundamental conception is concerned with reactions of cells to various stimuli including those which arise from the presence of microbes. Such stimuli often demand reactions in cells with other specialized function and the failure to respond adequately may be due to many factors such as previous overactivity or underactivity, interference with blood supply, faulty diet, and similar general nutritional deficiencies. Normally, the cells of the body are resistant to bacterial infection and the failure in this resistance is in many cases simply a failure to react—a fatigue in the normal defence mechanism.

The “common cold” which so often follows slight exposure; even such trivial things as taking a bath or washing the hair under favorable circumstances, and which contributes so much to the frequency of exacerbations in asthma was discussed by Holman¹⁴. He believed the “cold” is a question involving a consideration of the factors influencing the circulatory mechanism. A capillary system capable of adapting itself to such emergencies will undoubtedly usually prevent such infections. Disturbances of the capillaries may result from variations of atmospheric heat and cold, humidity, changes in the composition of the blood, heat regulation and the dangers of excessive water intake, and reflex effects. These facts partially explain such clinical findings as the dyspnea from increased pulmonary ventilation due to poor evaporation and radiation from the skin, and the deleterious effects sometimes observed in excessive water intake and at times a slight improvement in the asthmatic state resulting from a restriction of fluids.

Some other conditions favoring the infecting organism are Vitamine A deficiency; edema and hydration which develop under restricted protein and a high carbohydrate diet, mild acidosis; lack of beneficial tonic effects of regulated exercise, general fatigue, constipation, worry, loss of sleep, and similar strains.

Coexistent sinus disease has been shown to be of tremendous significance in asthma.

Aside from the purely allergic reaction, excessive ingestion of food with fermentation and putrefaction is so closely related to so many asthmatic seizures that there must be some chemical or toxic basis for this association. The chemical reactions of the body are entirely dependent upon the food intake and a temporary alteration of tissue pH may follow the production of organic and inorganic acids in the bowel. Fisher¹³ demonstrated the increased imbibition of hydrophyl colloids produced by variation in pH. MacLeod²⁴ showed that changes in tissue pH may occur without any change in the pH of the blood stream. The action of histamine, a putrefaction product, upon the capillaries is sufficient to cause local edema and Eustis^{11,12} observed clinical asthma and urticaria resulting from amines of intestinal origin. McCarrison²³ felt that the functional perfection of the adrenal glands is dependent upon the balance of the food and upon the quality and quantity of its vitamins.

Sooner or later the individual develops a neurosis whereby he has a greater number of attacks because of the constant fear of the attack coming at an inconvenient time or of the embarrassment that might result; or of the pity and solicitations of his associates. Then too the asthmatic patient at times employs his condition as an excuse to avoid obligations and duties of various types.

THERAPEUTICS

In the treatment of attacks, I have given these patients sufficient adrenalin to afford complete relief. Each patient was taught to give himself a hypodermic injection and this was of particular value because the patient himself could relieve attacks during the night and avoid loss of sleep. Atropine has been of

great value in the management of "colds." Each patient was instructed to use this drug as soon as the first sign of a "cold" appeared.

Prevention of attacks was attempted by control of the precipitating factors which included necessary surgery for sinus disease and very fortunately proper nasal surgery often gave temporary relief. Since both the secretions of the adrenal prevent nervous and muscle fatigue which predispose to asthma; since the cortico-adrenal secretion is so important in maintaining proper digestive function and since adrenalin remains the best measure in relieving asthma, it is fair to assume that the proper function of the adrenal is a necessary aim. It was demonstrated by Crowden and by Edmunds and Smith¹⁰ that rest restored adrenalin to a depleted medulla and the latter workers showed also the depleting effect of stimulating drugs. Crowden's experiments showed also that food prevented adrenal exhaustion by cold. Abundance of rest and careful administration of good food over a long period of time improved the body structure of all these patients so that bouts of asthma were fewer and less severe although the asthma did not disappear completely in any patient. The restricted mode of living is not a great difficulty to the patient, but a more vexing problem is to adapt the occupation to the patient's capacity. Dehydration of the nose by argyrol tampons was frequently used with some success. Dehydration of pulmonary tissue by marked reduction of fluid intake was employed in two patients with some temporary relief of symptoms, but this procedure alone was not sufficient to produce noticeable relief.

SUMMARY

In the causation of bacterial asthma one should remember the possibility of injury to the endocrine glands, especially the adrenals. Factors which cause capillary weakness often precipitate attacks of asthma because of turgescence of pulmonary vessels. Therapeutic measures designed to restore the function of the adrenal glands were beneficial but not curative to my patients with bacterial asthma.

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NOTES ON TECHNIC

A NOTE ON THE ADAPTATION OF FORMALIN-FIXED TISSUE FOR MALLORY'S AND WEIGERT'S STAINING METHODS

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Pathologists are constantly endeavoring to modify existing cytologic staining methods in an attempt to improve or simplify present technic, or else to introduce new methods for the better demonstration of cell structures. In a previous note* I described an adaptation to formalin-fixed material for Mallory's stains, and since that time an even more satisfactory modification has been adopted in our laboratory.

The formalin-fixed material is washed briefly in running water or ammonia water,¹ although both these procedures may be omitted. If there is much blood in the tissue, the ammonia water reduces the formalin precipitate on the stained preparations. The tissue is fixed in Zenker's solution for six to eight hours, and washed in running water for twenty minutes to half an hour. The tissue is placed overnight or longer in Weigert's secondary mordant for myelin sheath stains.² It is then embedded in paraffin and cut and stained in the usual way with any of Mallory's

* Kernohan, J. W.: A new modification of Mallory-Heidenhain's differential staining method and adaptation of formalin-fixed material for Mallory's stains. *Am. Jour. Clin. Path.*, 1: 399-403. 1931.

¹ Ammonia water is made by using 10 c.c. of tap water and adding approximately 60 drops of ammonia.

² Weigert's secondary mordant for myelin sheath stains:

| | | |
|--------------------------------|-----|------|
| Acetate of copper. | 5 | gm. |
| Chromium fluoride. | 2.5 | gm. |
| Acetic acid, 36 per cent. | 5 | c.c. |
| Water. | 100 | c.c. |
| Formalin. | 10 | c.c. |

stains. In the last year, I have used this method for peripheral nerves, spinal cord, and small pieces of brain tissue, and have found it equal to the original methods of Weigert. It gives as satisfactory results as the modification which I described previously, and it is also much more rapid, as it saves approximately four days in the procedure. The results obtained by this modification are superior to and more uniform than those obtained by treating sections of formalin-fixed material on a microscopic slide with Zenker's solution alone. It is equally valuable for all tissues of the body, and especially useful in studying the capillary loops and basement membrane of the glomeruli of the kidney by the Mallory-Heidenhain stain.

A NOTE ON DIRECT MATCHING OF BLOOD PRIOR TO TRANSFUSION

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(1) Draw a drop of blood from patient's finger tip to the 1 mark in a leukocyte counting pipette and follow with 5 per cent sodium citrate solution to the 11 mark.

(2) Mix thoroughly by shaking and transfer to small glass vial suitably marked for identification.

(3) Repeat for each donor.

(4) With an erythrocyte pipette, make a 9 to 1 combination of patient's and donor's blood by drawing blood from donor's vial to the first or 0.1 mark and patient's to the 1 mark at top of lower portion of the pipette.

(5) Transfer mixture to end of a glass slide and mix by alternate sucking and blowing three or four times. Add a small drop of saline and cover with cover slip.

(6) Now make a 1:1 dilution by drawing patient's blood from the vial to the 0.5 mark and donor's blood to the 1 mark. Mix and transfer to the other end of slide in same manner.

(7) Prepare a separate slide in this way for each donor. There is then a mixture of 9 parts of patient's blood to 1 of donor's at one end of slide and a 1 to 1 mixture at other end.

(8) Agglutination will show up both micro- and macroscopically in two or three minutes at room temperature.

(9) If no clumping occurs in fifteen minutes transfusion is safe.

(10) Transfusion is dangerous if agglutination is present in the 9 to 1 combination.

(11) If the 9 to 1 mixture is clear and the 1 to 1 shows agglutination, transfusion can be done but with caution; be ready to stop at once if untoward symptoms develop.



EDITORIAL

THE ERYTHROCYTE SEDIMENTATION TEST

We are indebted to Fahraeus for initiating observations on the sedimentation rate of erythrocytes when blood is mixed with anticoagulants and allowed to stand in tubes. Since his publication in 1918 a vast literature has accumulated. At first this dealt with methods and attempts at explaining the phenomenon, then with the test as applied to various diseases and more recently with critical analyses pointing out previous fallacies and setting up the limitations of usefulness of the test.

It is of course clear that the relation of the specific gravity and mass of the cell to the specific gravity of the plasma must determine the rate of fall but the factors influencing these reactions still remain obscure. To some the content of fibrinogen seems to be all important, to others the size of the cells, still others think the lipoid content of the serum is a factor. That the number of erythrocytes per unit of blood is of tremendous importance has been clearly shown by Gram, who made his correction in terms of hemoglobin content, by Meier and by Rourke and Ernstene on the basis of cell counts, and now more recently by Walton¹ who has clarified this consideration. He demonstrated a distinct relation between the rate of sedimentation and the number of erythrocytes showing that the rate increased as the number of erythrocytes decreased. This he observed experimentally by varying the concentration of cells with the addition or subtraction of the patient's own plasma. Further he has greatly simplified the procedure of performing the test and correcting for the number of erythrocytes.

Walton uses 3.8 per cent sodium citrate in a ratio of 1:10 with the blood. He advises a 6 mm. diameter tube and cautions that

¹ Walton, A. A. R.: The erythrocyte sedimentation test; a clinical and experimental study. *Quart. Jour. Med.* 2: 79-115. 1933.

the tube must be held vertically and during the hour should be at a temperature between 19° and 23°C.

It is evidently important to correct the test for erythrocyte count since a difference of 1,000,000 cells will often double the rate, hence uncorrected rates will give false results.

Based on a 5,000,000 erythrocyte count, no normal person will have a rate higher than 5.5 mm., and as a rule females have a higher rate than males. Normal processes that affect the rate are the menstrual cycle and pregnancy.

The test has suffered adverse criticism chiefly due to the fact that the earlier workers tried to make a specific diagnostic test of this reaction and it did not take long to break down this thesis. Many discarded the test and it is only recently that due to a clearer understanding of its limitations it is gaining advocates.

The view is now taken that the stable condition of the blood, attained by the age of one year, is lessened in relation to the severity of pathological processes regardless of their causes and that illness of any nature increases the sedimentation rate. As one becomes more ill the rate increases. Further, the rate increases often before other signs are manifested. In this light the test becomes of great value in the prognosis of disease, in indicating latent infections and in many problems of differential diagnosis. In separating neurotic patients from patients suffering with organic diseases the test is of great value, and in following the cause of acute infectious diseases and convalescent surgical patients, valuable warnings may be received even several days ahead of clinical signs or a decreasing rate may remove unfounded fears.

This very simple test should be more widely used for it frequently gives a quick answer to a difficult problem.

T. B. MAGATH.

NEWS AND NOTICES

THE TWELFTH ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

The Twelfth Annual Convention of the American Society of Clinical Pathologists will be held in Milwaukee June 9-12, with Headquarters at the Pfister Hotel. The Program Committee has planned an exceptionally fine program and it is hoped that it will be well attended. A particular feature is the Friday afternoon Symposium on "The Medico-Legal Postmortem" with the following subjects and speakers:

The Medico-Legal System of the United States. O. T. Schultz, Evanston, Illinois.

The Medico-Legal Autopsy. Charles Norris and A. V. St. George, New York City.

Postmortem Technic in Gunshot Wounds, Stabbing and Strangulation Cases. H. S. Martland, Newark, New Jersey.

Pathologic Anatomy in Cases of Drowning. E. L. Miloslavich, Milwaukee, Wisconsin.

The Rôle of Toxicology in a Medico-Legal Autopsy. A. O. Gettler, New York City.

Discussion. William D. McNally, Chicago, Illinois.

A Round Table Discussion dealing with the State Laboratories and laboratory technic will be held at the Pfister Hotel Friday June 9. The Annual Banquet will be Saturday evening and the Business Meeting on Monday.

Owing to the fact that the Century of Progress Exposition will be in full force the local committee has assumed that wives and children will accompany clinical pathologists, accordingly they have made arrangements for social features which will appeal to the family. This includes a boat trip on Lake Michigan on Sunday. Those who desire more information than was contained in the letter of April 11th, should get in touch with either the

members of the local committee or the chairman of the Program Committee.

Dr. Thomas Parran, Jr., Commissioner of Health, State of New York has just issued the new requirements for surgical pathologists in the State of New York laboratories. This follows the same general standards set up for directors and bacteriologists which include at least four years experience or training in the field subsequent to graduation. In addition to this, surgical pathologists are given a practical examination in the form of a series of tissue sections from which they must make diagnoses. The Public Health law establishing these conditions was based on the theory that numerous reports of tissue were being made by pathologists who lacked the proper qualifications and that this was a serious matter since such an error was likely to result either in a needless operation or in the failure to operate when the patient needed it.

Owing to the fact that so few abstracts of papers were submitted, it was decided to omit their publication in this issue of the JOURNAL.

THE NEUTROPHIL IN PERNICIOUS ANEMIA*

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There is comparatively little in American medical literature on the neutrophilic features of pernicious anemia, and the aid which such features can furnish in diagnosis. DeCosta,⁸ and Cabot,⁵ stated that in many cases the neutrophilic leukocytes do not manifest abnormalities in size and staining reaction, frequently being much smaller than normal. Arneth,¹ published his first paper dealing with variations in the number of lobes of the nuclei of the polymorphonuclear neutrophils in health and disease, but it was not until 1907 that he² discovered in two cases of uncomplicated pernicious anemia that there was a definite and marked shift to the right in the neutrophilic leukocytes. Decastello,⁹ noted the abnormal number of lobes in the neutrophil nuclei in a case of pernicious anemia after splenectomy, and Brosämlen,³ noted that there was a shift to the right in cases of pernicious anemia during remission. In the course of a study of the Arneth count, Briggs,⁴ was the first in this country to find that in ten of twelve cases of pernicious anemia there was a decided increase in the number of lobes in the nuclei of neutrophils. He also felt that there was no relationship between the severity of the anemia and the degree of the shift.

Naegeli,¹¹ in a paper on early diagnosis of pernicious anemia was able to demonstrate marked segmentation of the neutrophil nuclei. Piney,¹² stated that there is morphologic evidence of defective function of bone marrow in that hyaline leukocytes are always reduced in number and that there are always a varying number of polymorphonuclear leukocytes of large size with hypersegmented nuclei.

* Read before the Eleventh Annual Convention of the American Society of Clinical Pathologists, New Orleans, Louisiana, May 6-9, 1932.

Cooke,^{6,7} reported twenty cases of pernicious anemia studied by a modification of the Arneth count and found a right shift in all of them.

Neuberger,¹² stated that a left shift occurs only in pernicious anemia with complications. He also came to the conclusion that the return of the right shift to normal could not serve as a criterion for a therapeutic result, since this return may occur only a long time after there has been marked improvement, and even after there has been complete remission.

Jedlička and Beranek, presented a report on thirty-six cases in which lobes were counted and believed that a shift to the left is more characteristic of pernicious anemia than a shift to the right. They emphasized the presence of cells in which the nuclear skein is markedly elongated as well as pyknotic in appearance. They term this characteristic "pathological hyperpolymorphism" and place it next to increased segmentation in significance as a diagnostic aid.

Watkins and Berglund,¹⁴ found that the neutrophil of pernicious anemia remained after the blood had returned to normal. They did not determine if this deviation from normal ultimately disappeared, but felt that it was an aid in the diagnosis of pernicious anemia after the blood had become practically normal. Following the introduction of treatment by liver, Fleming¹⁰ studied a case of pernicious anemia in regard to the lobulation of the neutrophil, both before and after treatment with liver as well as in relapse. He showed that under adequate treatment the increased lobulation disappeared, but that as soon as treatment was discontinued, this change again became apparent.

The features of the blood picture in pernicious anemia may be divided into those changes which occur in erythrocytes and those which occur in granular leukocytes. The changes in erythrocytes may be further subdivided into the degenerative and regenerative. The features which have been emphasized in the past are poikilocytosis, macrocytosis with certain of the cells consisting of the large oval type, hyperchromasia, anisocytosis, polychromatophilia, nucleated red cells of the normoblast and megaloblast series, and basophilic stippling. Many observers have em-

phasized particularly the significance of the megaloblast in the diagnosis, and insist on its presence before making a positive morphologic diagnosis. The question of derivation and differentiation of megaloblasts and normoblasts is still controversial.

The outstanding morphologic feature of pernicious anemia is the macrocytosis. This is most striking since it is present in every field in a well-made smear. Changes in the neutrophil are revealed only by more detailed study of the smear, as in cases of leukopenia and lymphocytosis it may be necessary to make the

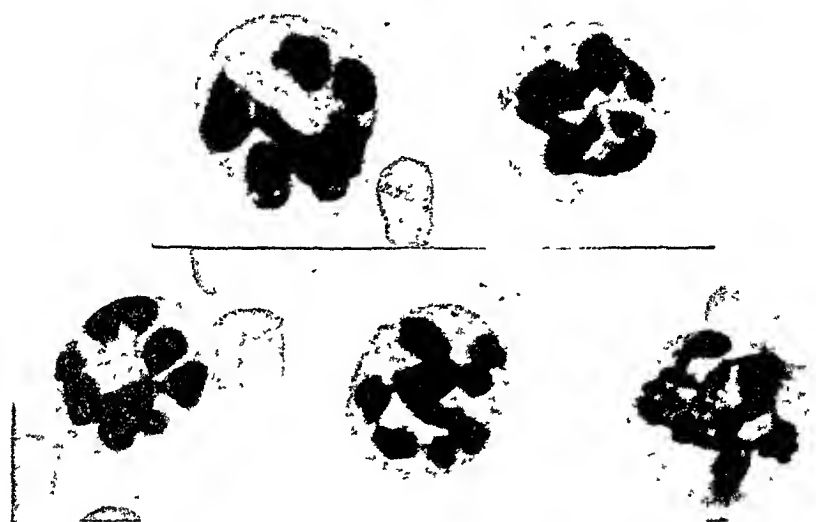


FIG. 1. NEUTROPHILS SHOWING INCREASED SEGMENTATION AND POLYMORPHISM OF THE NUCLEUS

equivalent of a differential count of 300 leukocytes in order to bring out the change.

Since in the observation of patients under treatment all of the foregoing features tend to disappear, and since many early cases may pass unrecognized, one should make use of all diagnostic criteria. We have studied fifty cases in which an unequivocal clinical diagnosis of pernicious anemia was made, in all of which one or more of the morphologic features of the disease were present. All of these patients had achlorhydria. In addition three cases were studied in which there was definite macrocytosis but

in which an adequate quantity of free hydrochloric acid was demonstrated. An average was taken of 100 neutrophils counted by each of us. The criterion laid down by Cooke, that only those lobes should be counted which are definitely separated by thin thread-like strands, has been applied. The difference in criteria of various observers has undoubtedly given rise to the variations in counts of lobes which have been reported. In using the Cooke criterion, neutrophils with marked polymorphism may at first glance appear to be many lobed, but after careful study thread-like filaments will not be seen. The polymorphism as well as increased segmentation is shown in figure 1. Although Schilling recognized the occurrence of a right shift in pernicious anemia, his hemogram in these cases does not actually reveal such a change

TABLE 1

COUNTS OF LOBES IN THREE CASES OF MACROCYTOSIS AND FREE HYDROCHLORIC ACID: DIAGNOSIS INDETERMINATE

| NUMBER | HEMO- GLOBIN | ERYTHRO- CYTES | LEUKO- CYTES | LOBES | | | | |
|--------|-----------------|-------------------|-----------------|---------|---------|---------|---------|---------|
| | | | | Class 1 | Class 2 | Class 3 | Class 4 | Class 5 |
| | <i>gm</i> | <i>millions</i> | | | | | | |
| 1 | 9.6 | 1.96 | 4,800 | 8.0 | 15.0 | 36.5 | 24.5 | 16.0 |
| 2 | 9.7 | 2.12 | 6,000 | 23.5 | 27.5 | 30.5 | 10.5 | 8.0 |
| 3 | 12.0 | 2.48 | 5,500 | 18.5 | 25.5 | 37.5 | 15.0 | 3.5 |

since all cells with a greater number of lobes would be included under the heading "segment forms." Cooke demonstrated that among ninety normal patients the average distribution of lobes was as follows: in class 1, ten cells, in class 2, twenty-five cells, in class 3, forty-seven cells, in class 4, sixteen cells and in class 5, two cells. The occurrence of more than three cells, in class 5, in the normal subject is extremely uncommon. In table 1,* all cells with five or more lobes are included under class 5.

Although for the most part our results agree closely with those of other observers, we cannot confirm the observation that all cases of pernicious anemia manifest increased lobulation or a

* The authors can furnish, upon request, a table of values observed in fifty patients.

right shift in the neutrophils. The usual picture seen is that of macrocytosis with a right shift in the neutrophil. In some of these cases there was a definite tendency to a left shift, although no complicating infection could be found which might give rise to such a change. Our average values for class 1 are considerably higher than those of Cooke. In cases 7 and 28 in which there was no right shift there was definite macrocytosis. On the other hand, two cases, 1 and 49, are included in which there was no macrocytosis but in which there was a definite increased segmentation in the neutrophils. The persistence of macrocytosis, as well as right shift, is demonstrated in case 47 which apparently had been adequately treated. The presence of such changes raises the question of whether the treatment was really adequate, and if it would not be advisable to increase the dosage of active material in an attempt to bring about a normal blood picture. We have seen a few instances in which a positive blood picture persisted for more than a year after institution of treatment with normal blood counts and disappearance of all symptoms. Usually under adequate treatment, the blood picture returns to normal so that no evidence of pernicious anemia can be found. In contrast to the cases mentioned in which the blood picture showed the morphologic changes of pernicious anemia, it is significant that when there is peripheral neuritis of the pernicious anemia type or combined sclerosis with little if any anemia, morphologic examination of the blood smear frequently is not of aid.

Counts of lobes are presented in three cases in which there were morphologic conditions ordinarily associated with pernicious anemia, but in which there was adequate free hydrochloric acid on analysis by test meal. In two of these cases determinations of volume index gave values of 1.13 and 1.45, respectively. In case 2 of this group the response to Lilly's liver extract 343 was excellent. For more than a year the patient had taken an average of twenty-four tubes of this preparation each month without relapse and in the last three months he has remained in excellent condition in spite of the fact that he has not had any treatment directed toward the anemia. In case 1, practically normal blood counts are maintained as long as the patient continues to take

liver extract. One patient (case 3) is under observation at the present time so that data relative to response to treatment are not available. Except for diarrhea in case 2 none of the cardinal symptoms of pernicious anemia was present, and neurologic examination gave entirely negative results.

It is evident from our results that the occurrence of neutrophils with more than five lobes bears no relationship to degree of anemia or to number of leukocytes. It is necessary to emphasize that diagnostic features of the neutrophil in pernicious anemia are not confined to lobulation of the nucleus but that the finer structure, such as elongation of the nuclear skein and connecting strands, thinning of the lobes and slight condensation of chromatin in the lobe, are of significance. In cases in which there is no macrocytosis the finding of right shift or hyperpolymorphism of the neutrophil nucleus may be an aid in the diagnosis.

SUMMARY

Fifty cases of pernicious anemia, in all of which there was achlorhydria, have been studied with reference to the lobulation of the neutrophil. Increased segmentation in the neutrophil is the rule, although cases do occur in which there is a left shift and absence of the hypersegmented neutrophil.

In three cases in which there were morphologic changes of pernicious anemia in the presence of adequate quantities of free hydrochloric acid, but in which other characteristic symptoms of pernicious anemia were absent, no essential differences could be found from the former group.

There is no invariably pathognomonic blood picture of pernicious anemia, since the same changes may be demonstrated in sprue and occasionally in an indeterminate group of anemias.

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SUGAR METABOLISM AND BLOOD STUDIES FOLLOWING VAGOTOMY

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In the course of experiments on the effect of section of the vagus nerve on gastric secretion,⁵ it was found that the majority of the animals following vagotomy lost a considerable amount of weight and some succumbed with convulsions. At that time we were unable to account for the deaths of these animals but we did discover that when they were fed with milk instead of the usual laboratory diet they invariably increased in weight and never manifested convulsive seizures. Vanzant¹¹ had also noticed that some vagotomized dogs lost weight but when fed with fresh meat and milk they regained their normal health.

No adequate explanation has been offered for this phenomenon but it occurred to us that there may have been some factor in the milk which prevented convulsions and loss of weight and which promoted well-being. For this reason it was decided to study the chemistry of the blood in a group of animals in order to determine whether variations occurred in the calcium, phosphorus, sugar, urea nitrogen, carbon-dioxide combining power and chlorides. So far as we have been able to ascertain, no one has satisfactorily studied the complete blood chemistry in vagotomized dogs.

BLOOD CHEMISTRY STUDIES

For this experiment ten dogs were utilized. In eight the right vagus was severed and in two, both vagi were cut just below the diaphragm. Blood chemistry studies were made before this operation was performed in order to establish a normal. The normal varied in different dogs as well as in the same dog and for

that reason the results may be less conclusive than otherwise. After vagotomy, blood for chemical determinations was taken at intervals over a period of four months in some instances. All values were obtained after a twenty-four hour fast.

Table 1 gives the average values in each of the eight vagotomized dogs before and after the operation. In dogs 2 and 4 the

TABLE 1
AVERAGE BLOOD CHEMICAL VALUES*

| DOG | SUGAR | UREA NITROGEN | CO ₂ COMBIN- ING POWER | CALCIUM | PHOSPHORUS | CHLORIDES |
|-------------------------------|-------|------------------|--------------------------------------|---------|------------|-----------|
| A. Before unilateral vagotomy | | | | | | |
| 1 | 92 | 22.8 | 47.5 | 10.5 | 4.5 | 493 |
| 2 | 82 | 40.5 | 38.7 | 10.4 | 5.3 | 505 |
| 3 | 79 | 20.8 | 45.3 | 10.0 | 6.3 | 501 |
| 4 | 72 | 11.2 | 52.8 | 11.3 | 5.8 | 504 |
| 5 | 69 | 14.8 | 47.5 | 10.7 | 3.3 | 486 |
| 6 | 73 | 8.9 | 57.6 | 10.3 | 3.0 | 465 |
| 7 | 82 | 9.5 | 48.1 | 10.4 | 3.1 | 536 |
| 10 | 85 | | 52.2 | 11.6 | 6.6 | 556 |
| B. After unilateral vagotomy | | | | | | |
| 1 | 30 | 28.0 | 21.1 | 10.6 | 12.3 | 592 |
| 2 | 73 | 17.8 | 44.6 | 10.6 | 5.3 | 523 |
| 3 | 83 | 26.7 | 47.7 | 9.7 | 5.0 | 490 |
| 4 | 76 | 20.3 | 48.9 | 9.6 | 4.8 | 502 |
| 5 | 76 | 13.3 | 49.9 | 8.8 | 4.5 | 504 |
| 6 | 84 | 19.5 | 48.5 | 9.7 | 4.7 | 552 |
| 7 | died | | | | | |
| 10 | 84 | 19.6 | 48.0 | 10.7 | 5.0 | 453 |

* All values are expressed in mgm. per cent except CO₂ combining power which is in volumes per cent.

urea nitrogen varied over wide limits and in dog 4 there was also a decrease in calcium and phosphorous values after vagotomy. In dog 5 the calcium and phosphorus were lower after vagotomy while the chloride value was somewhat increased. In dog 6 the urea nitrogen values were increased after vagotomy (in dog 2 they were lowered, while in dog 4 they were increased), the calcium

value was lowered while the phosphorous value was increased and the chloride value increased. Dog 1 showed a markedly reduced sugar (hypoglycemic shock), the carbon-dioxide combining power was greatly reduced, the phosphorous value was greatly increased and the chlorides were also somewhat increased. Dog 7 died and dog 3 showed little change before and after vagotomy. Only dogs 1, 4 and 5 had convulsions (see below) in this series. Although there were definite changes in certain of the blood chemical values they were not consistent and whereas in one case the urea nitrogen was lowered after vagotomy, in another it was increased. It would therefore be difficult to draw any definite

TABLE 2
AVERAGE BLOOD CHEMICAL VALUES*

| DOG | SUGAR | UREA NITROGEN | CO ₂ COMBIN- ING POWER | CALCIUM | PHOSPHORUS | CHLORIDES |
|---------------------------|-------|------------------|--------------------------------------|---------|------------|-----------|
| Before bilateral vagotomy | | | | | | |
| 33 | — | — | — | — | — | — |
| 9 | 92 | — | 54.8 | 12.0 | 7.7 | 515 |
| After bilateral vagotomy | | | | | | |
| 33 | 66 | 13.6 | 49.5 | 8.6 | 4.2 | 466 |
| 9 | 84 | 17.6 | 48.0 | 10.0 | 4.6 | 526 |

* All values are expressed in mgm. per cent except CO₂ combining power which is in volumes per cent.

conclusions from these results. In the three dogs in whom convulsions occurred number 1 showed hypoglycemia, number 4 an increase in urea nitrogen, and number 5, a decrease in calcium and phosphorous values. Perhaps the most striking of these changes is the extreme hypoglycemic reaction of dog 1; in this dog the blood happened to be taken just at the time of the convulsion which resulted in death. The values obtained in the study of dog 3 represent an example of those usually observed. In this animal there was quite a variation in the urea nitrogen. The sugar values remained within normal limits. The phosphorous values varied between 4.3 and 6.45, the calcium between 8.4 and 10.32

and the chlorides between 453 and 536. These values, however, were considered within normal limits. This dog had no convulsions but did lose weight. All values, of course, are obtained under basal conditions after a twenty-four hour fast.

In two dogs bilateral vagotomy was performed. The effect of this operation upon the blood chemistry is shown in table 2.

In dog 9 there was a reduction in calcium and phosphorus after vagotomy. Unfortunately satisfactory normals were not established in either dog 9 or 33. Nevertheless it is interesting that the normal calcium and phosphorus was so high in dog 9. Both of these dogs lost weight progressively and finally died, dog

TABLE 3
BLOOD CHEMICAL STUDIES* ON DOG 1

| DATE | SUGAR | UREA NITROGEN | CO ₂ COMBIN- ING POWER | CALCIUM | PHOSPHORUS | CHLORIDES |
|-----------|-----------------------------------|------------------|--------------------------------------|---------|------------|-----------|
| 1/26/32 | 88 | . | 38.5 | 10.2 | 4.0 | 494 |
| 1/28/32 | 110 | 21.4 | 44.3 | 10.4 | 4.5 | 524 |
| 2/ 2/32 | 79 | 24.1 | 59.8 | 10.8 | 5.0 | 462 |
| 2/ 3/32 | Vagus section (sub-diaphragmatic) | | | | | |
| 2/ 5/32** | 30 | 28.0 | 21.1 | 10.6 | 12.3 | 592 |

* All values are expressed in mgm. per cent except CO₂ combining power which is in volumes per cent.

** Died in convulsions, heart's blood obtained immediately. Heart's blood was frequently used during these studies and corresponds closely with values obtained on jugular blood.

33 in nine days, dog 9 in eighteen days. The results of chemical tests done after vagotomy would be considered within normal limits. These results although meagre, would not support a "blood-chemistry" explanation of these deaths.

As has already been recorded, the above experiments were carried out over a period of four months and observations were made at frequent and varying intervals. The first observations were made shortly after vagotomy and these were followed at intervals over a period of four months.

Although convulsions occurred in dogs 1, 4 and 5 all of them lost weight. However, only in dog 1 did death result while the animal was in convulsions. A detailed blood-study on dog 1

revealed the striking hypoglycemia which was not observed in any of the other animals. Table 3 illustrates the blood findings in this animal. Note the remarkably low value of carbon-dioxide combining power and the high value for phosphorus.

LITERATURE

Many years ago, Pavlov⁹ came to the conclusion that it was "definitely settled that the vagus is the secretory nerve of the pancreas" and he also remarked

"how similar the nervous connections of the stomach and pancreas have proved to be; the innervation of the one is in every respect a copy of the other. It is therefore permissible to fill up gaps in our knowledge of the scheme in one case by analogy from the other. We cannot doubt, for example that secretory fibres for the stomach are present not only in the vagus but also in the sympathetic."

According to most anatomists the right vagus nerve sends branches to the pancreas but the left does not. The important fact is, however, that the vagus nerve may by its innervation of the stomach, pancreas, etc. alter, at least temporarily and theoretically the chemistry of the blood or the blood's ability to react to certain chemical changes. If this did occur it might be ascertained by insulin tolerance tests (see below).

Several years ago Clark² investigated the influence of the vagus on the islets of Langerhans. He recalled the work of Banting and Gairns which indicated that the secretion of insulin is not necessarily dependent on the nerve supply to the islets of Langerhans but he did not believe it unreasonable to suppose that the nervous system may influence the secretion of insulin. Furthermore, he remarked that the nerve supply to the islets of Langerhans is conveyed, mainly, if not entirely, in the right vagus and that MacLeod and his co-workers had produced hypoglycemia by stimulation of the right vagus in the neck. In Clark's experiments the glucose tolerance test tended to show that sufficient insulin could be produced to meet an emergency without vagal control. In general, Clark's results suggested that stimulation of the vagus caused a secretion of insulin with hypoglycemia. Later he³ sectioned the right vagus in the neck and found that the

animals (rabbits) went along quite all right for many months showing no change in blood sugar level. "It is clear that the essential function of the islets in the metabolism of sugar is carried on in spite of the nerves having been cut." He was unable to demonstrate decreased sugar tolerance following right vagotomy within a period of two months. On the contrary, in a few cases an increase in tolerance was suggested. He concluded that after vagotomy the sugar tolerance is for some weeks increased and not diminished but that the increased tolerance after vagotomy gradually declines and is later followed by diminished tolerance. In a subsequent paper, Clark⁴ concluded that

"section of the right vagus above or below the diaphragm in cats under amytal anesthesia produces an immediate fall in blood sugar, the degree of which varies with (a) the initial blood-sugar level and (b) the glycogen reserve. It is suggested that this effect is produced by cutting off tonic inhibitory influences which control insulin secretion."

MacLeod⁷ cautioned that

"since in all these animals (rabbit, cat and dog) a certain degree of variation is observed, even when the conditions of feeding of housing and of preliminary treatment are standardized as far as possible, it is necessary to know, not only the average, but also the extent of deviation."

In normal unanesthetized dogs the blood sugar values vary from 0.057 to 0.088 per cent according to the majority of investigators.

After insulin the picture of hypoglycemia is rather characteristic. These symptoms so closely resembled those shown by our animals dying in convulsions after vagotomy that it is important to describe them here. In the dog

"the first signs are usually very rapid breathing, restlessness and general hypersensitivity, coupled with loss of interest in its surroundings and indifference to the attention of its master. Muscular twitching then becomes evident, and the sphincters may relax. Barking is often a prominent symptom, and there may be salivation and frothing at the mouth. At this stage, or it may be as the first symptom, convulsions, not unlike those seen in the rabbit, may supervene, and between them the animal lies on its side evidently unconscious, showing violent twitchings of the musculature, amounting almost to tetany. The rates of

breathing and of the pulse increase. Inspiration is usually short and jerky and inspiratory tetanus not infrequent, so that artificial respiration may have to be applied. Attempts to get on its feet are often the cause for convulsive seizures, and during recovery the muscles of the extremities, particularly the anterior, are seen to have entirely lost their power of coördinate action."⁷

Although convulsions may be due to acidosis, alkalosis, disturbed calcium-phosphorous ratios, to hypoglycemia or hyperglycemia, gastric tetany (loss of chlorides) et cetera, Hosoi and Alvarez¹ some years ago were convinced, as a result of their work, that both the vagus and splanchnic nerves served largely as brakes to the bowel and that without these brakes the bowel tends to respond to every stimulus and the animals often die of diarrhea and inanition. Our dogs manifested both symptoms but usually seemed to die of the latter and it is possible that this disturbed physiological mechanism may ultimately be found to explain the entire picture. Nevertheless, hypo- and hyper-insulin states have recently attracted so much attention in medicine that this explanation must be given sufficient consideration.

It is interesting that La Barre⁶ established an average blood sugar level of 73 mgm. per cent in normal dogs fasting forty hours and found the value to be practically the same after vagotomy above the diaphragm. He also noted that after the administration of glucose there was an earlier return to the original level in the vagotomized animals and that the fall below the original level was more marked than in the normal animals. Later Quigley, Halloran and Barnes¹⁰ reported that chronic vagotomy did not reduce the sensibility of the mechanism which is stimulated by glucose but on the contrary seemed to make it more irritable. For example, they observed that vagotomized animals metabolized "administered glucose" more rapidly than the normal animals.

SUGAR TOLERANCE STUDIES

The record of dog 1, lead us to investigate the sugar metabolism in a series of dogs. The plan was to investigate the response of normal animals to insulin and glucose (glucose tolerance test) and then to examine the same animals after vagotomy. In eight

normal dogs a sugar tolerance test was performed (25 grams of sugar were given intravenously to each dog of approximately equal weight). In five of these the tolerance curve was normal while in the remaining three (dogs 16, 17 and 18) the blood sugar continued to be high (174–250 mgm. per cent) even after two and one-half hours. Six of these dogs in addition to several others were vagotomized and glucose tolerance tests were repeated several times. The same variations which occurred in the normal animals also occurred in the vagotomized ones and one is inclined to believe after studying such results that vagotomy is probably not a factor in these variable results and that they more likely depend upon some underlying disturbed metabolic phenomenon occurring in each animal. Seven animals (dogs 14 (1), 14 (2), 15, 6, 5, 10, 1b) gave essentially normal tests. Dog 17 gave an abnormal response in each of three tests. Dog 16 responded normally in one test, abnormally in two others; dog 11 responded normally in one test, abnormally in another. Another striking observation is the fact that dog 14 (2) had a blood sugar of 200 mgm. per cent before glucose was given and that dog 17 on one occasion had a blood sugar of 84 mgm. per cent as a normal while in two other instances it was too low to read. Similarly dog 11 had a blood sugar of 76 mgm. per cent in one instance and less than 20 mgm. per cent in another. Notwithstanding these variations the dogs appeared to be normal; there were signs neither of hypo- nor hyper-glycemia.

It had previously been noted that dogs may respond differently immediately after vagotomy as compared with those cases in which months have elapsed since vagotomy. This aspect of the problem was studied and though many variations in sugar values were noted, yet, on the whole, the responses were not significantly different. Most of the dogs returned to normal, some did not; recent and old vagotomies seemed to play no important rôle in sugar metabolism. For example, dogs 5, 6, and 10 had been vagotomized three and a half months before the glucose tolerance tests were performed; dogs 16 and 11 one month before, and dogs 16, 17, 15, 14 (1) and 14 (2) were examined one, two and three weeks after right vagotomy. Dogs 5, 6, and 10

responded normally, dog 16 responds normally but dog 11 has a variable response; of the remaining animals, dogs 14 (1), 14 (2), and 15 responded normally and 16 and 17 did not. It is permissible to conclude that vagotomy may have an occasional immediate disturbing effect but this is never permanent according to these experiments. It seems that the body adjusts itself to vagus section and manages to function normally despite the loss of vagus innervation.

INSULIN STUDIES

In five vagotomized dogs and in three unoperated animals, 12 cc. of insulin (U 20) were injected intravenously in order to determine its effect on sugar metabolism. This dosage of insulin was chosen after gradual increases in insulin were given. This amount was found to produce a rather rapid hypoglycemia with its concomitant symptoms. The blood sugar levels varied similarly in all eight of the animals but in general, the vagotomized dogs seemed weaker and more subject to convulsive seizures and pareses. Three of the vagotomized dogs reacted with incoördination and weakness; one had convulsions, one became very quiet after insulin administration and was evidently profoundly parietic, one remained well even though the chemical determinations were identical with those in the other vagotomized dogs. Of the unoperated animals one remained in good condition, one became weak and unsteady and one went into convulsions but recovered quickly upon the intravenous administration of glucose.

AUTOPSY STUDIES

Autopsies were performed to verify vagus nerve section as well as to discover pathological changes. There were no striking gross changes. The bilaterally vagotomized dogs (9 and 33) showed in the first case a hemorrhagic area 2 cm. in diameter on the greater curvature just above the pylorus and in the latter a chronic passive congestion of the liver, lower lobes of both lungs, spleen and kidney and small hemorrhagic areas throughout the stomach. The other dogs showed no remarkable abnormalities.

In each instance, however, specimens for microscopic examination were obtained from the liver, spleen and from three portions of the pancreas and in all of these microscopic examination revealed no significant changes. It is interesting in this connection to mention the work of Mattioli* who injected dextrose and epinephrine into dogs and found, on histological examination of the pancreas, an increase in the number and size of the islands of Langerhans. When insulin was administered in addition to the dextrose a mild hyperplasia in the endocrine part of the pancreas was noted. The author concludes that the endocrine tissue of the pancreas is the principal factor in the mechanism of glycoregulation. These experiments are not really comparable to ours since vagus section was not done.

CONCLUSIONS

(1) In vagotomized dogs changes occur in the blood chemistry values but they are so inconsistent that their significance becomes dubious.

(2) The sugar tolerance test yielded variable and similar results in both the normal and vagotomized dogs. Some normal dogs failed to give normal glucose tolerance curves and many vagotomized dogs did respond normally.

(3) Vagotomy is probably not a factor in the production of these inconsistent reactions to glucose administration.

(4) The minor changes following upon vagus section disappeared quickly; the animals, after three months, responded normally in every way. We were unable to demonstrate any constant increased irritability of the mechanism stimulated by glucose administration nor were we able to conclude that there was an increased sugar tolerance test for the first few weeks after vagus section which later is followed by a diminished tolerance. The longer the studies were carried on (eight months in some cases) the more we were convinced that almost any reaction might be obtained.

(5) The blood sugar values following insulin administration were essentially the same in both normal and vagotomized dogs. Even though there were no significant changes in the sugar values,

nevertheless the vagotomized animals were more apt to manifest symptoms in the nature of weakness, nausea, vomiting, pareses, and convulsions.

(6) Autopsy studies did not disclose any noteworthy pathological changes. Vagotomy did not result in any histological abnormalities in the pancreas.

(7) The convulsions and loss of weight in these dogs can not be explained by demonstrable changes in the blood chemistry nor can they be based upon consistent abnormalities in sugar metabolism. It seems that a definite metabolic disturbance is accountable for this phenomenon and that withholding food certainly plays an important rôle in the production of these symptoms. However, other factors as yet undetermined may be concerned in their development and further studies must be made to discover them.

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LEPTOSPIRAL JAUNDICE

A REPORT OF TWO CASES

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The wide geographical distribution of rat carriers of *Leptospira icterohemorrhagiae* as determined by Jobling and Eggstein,⁵ Otteraaen,⁹ Ridlon,¹⁰ Wadsworth et al.,¹⁵ and Langworthy and Moore,⁶ makes the report of these human cases of leptospiral jaundice from southern California desirable. Human cases have now been recorded from Canada to Tennessee and from the Atlantic to the Pacific coasts.

Following the work of Inada and his co-workers,⁴ authentic cases occurring in North America were reported by Wadsworth et al.,¹⁵ Sailer,¹¹ McDowell,⁷ Bates,¹ Towler and Walker,¹⁴ Cushing,² Hayman and Lynch,³ and Mulholland.⁸ The present cases are the ninth and tenth in American literature.

Sellards and Theiler¹³ in 1927 advanced evidence to indicate that *Leptospira icterohemorrhagiae* and *Leptospira icteroides* were identical. Sawyer and his co-workers¹² in 1930 presented evidence indicating that neither African nor American yellow fever are caused by leptospiras. The similarity of the clinical picture in leptospiral jaundice and yellow fever with its consequent mistakes in diagnosis, explains the error of Noguchi in ascribing the leptospira of one disease as the etiologic agent of the other.

CASE HISTORIES

1. The patient, a white Canadian war veteran forty-two years of age, without stated occupation, entered the Los Angeles County General Hospital, Nov. 18, 1928. He was so sick and uncoöperative that but a very meager history could be obtained. He had been ill for two weeks with high fever which subsided with the onset of jaundice five days prior to admittance. He was deeply

jaundiced. He had had generalized abdominal pain at times and had vomited frequently. He had not accepted food for several days, was partially demented, and could not seem to talk above a whisper. Subsequent questioning of a sister revealed the fact that just prior to the present illness he had been cleaning up an old deserted carpet factory.

A previous history in the same hospital revealed the information that there was X-ray evidence of a small calculus at the lower pole of the right kidney, and one at the uretero-pelvic junction of the left, with pyonephrosis. His blood Wassermann at that time was negative; the blood chemistry within normal limits. The stone on the left side was removed one year prior to the present illness, since which time he had experienced a bettering of his general health.

Examination revealed him to be well developed and well nourished, acutely ill, and at times tossing about. The sclerae were yellow. Normal pupillary reactions were present. The mouth was dry; the lips cracked; the tongue coated and swollen. No râles were heard in the lungs. The heart rate was rapid: the blood pressure 130/70. The abdomen was somewhat distended and tympanitic though rigidity and tenderness were present. There was a suggestion of a mass in the gall bladder area. The extremities were negative except for a sluggish patellar reflex.

Laboratory findings: Hemoglobin, 80 per cent; leukocytes, 14,250 per cu. mm.; polymorphonuclear neutrophils 94 per cent; large lymphocytes 5 per cent; transitionals 1 per cent. Blood chemistry: Sugar, 131 mg. per cent; non-protein nitrogen, 162; creatinine, 3.2; uric acid, 7.1 mg. per cent, respectively. Icteric index, 28.5; Van den Bergh test, direct: positive; indirect: 2.5. Urine: coffee colored, alkaline, specific gravity, 1.016, no sugar present, but a trace of albumin. Bile, four plus. The microscope revealed a few hyaline and granular casts, and an occasional red blood cell cast, as well as clumps of leukocytes.

Progress: A diagnosis of "suppurative cholecystitis with cholangitis and hepatitis" was made, and the gall bladder drained under a local anesthetic. The gall bladder was found greatly distended, thinned, and adherent to all surrounding structures. On incision there was free drainage of bile through the gall bladder. Smear from the gall bladder contents showed pus cells and many gram positive cocci. On culture a staphylococcus and *Esch. coli* (*B. coli*) were isolated.

Following the operation considerable blood drained through the tube inserted into the gall bladder. Some of this clotted and obstructed the tube. His condition became progressively worse. Death occurred four days after admission.

The autopsy was performed five hours after death and prior to embalming.

The lungs showed evidence of purulent bronchitis and bronchopneumonia in the lower lobes. The heart was not materially enlarged, and aside from bile staining of the serous surfaces there was no evidence of pathology.

The liver was slightly enlarged but retained an essentially normal appearance on cut section. The gall bladder was greatly distended and contained clotted blood. The common and cystic ducts were patent. There were no findings to

indicate an obstructive jaundice. The pancreas showed some degeneration. The spleen was rather large but did not show material gross evidence of pathology.

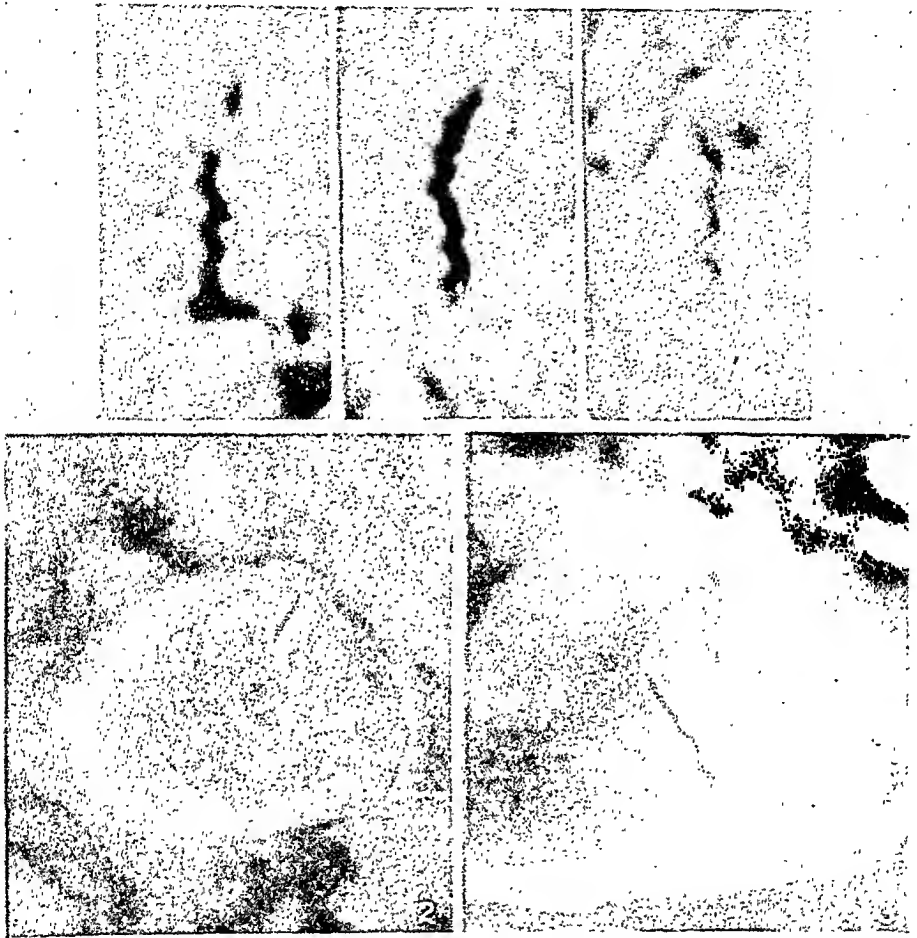


FIG. 1. THREE LEPTOSPIRAS FROM A LEVADITI IMPREGNATION OF THE KIDNEY
IN CASE 1
(5500 diameters)

FIG. 2. ORGANISM WITH HOOKED EXTREMITY IN KIDNEY TUBULE OF CASE 2
Levaditi stain (1500 diameters)

FIG. 3. ANOTHER ORGANISM IN A KIDNEY TUBULE
Levaditi stain (3000 diameters)

The left kidney was bound down by adhesions at the site of a previous operation and moderate hydronephrosis was present. There was no evident reason for this hydronephrosis and it may be considered a residuum from the previously

removed calculus. The kidney substance was swollen and hemorrhagic. The right kidney showed rather severe hemorrhages throughout the parenchyma. A hydronephrosis on this side was caused by a small calculus at the ureteropelvic junction.

Rapid frozen sections of liver, pancreas and kidneys revealed petechial hemorrhages in the pancreas, and cloudy swelling and suppuration in the kidneys. The liver showed areas of round cell infiltration and some congestion as evidence of a hepatitis.

Because the jaundice could not be explained on a mechanical basis, and since the microscopic findings were consistent with a generalized infection, the death certificate was signed using "septicemia with hepatitis and jaundice (organism not discovered)," as the cause of death. The possibility of Weil's disease was considered and smears of the kidney made and stained by Fontana's method. These showed definite silver positive wavy forms strongly suggestive of leptospiras, with the one objection that the ends were usually seen to be clubbed or bulbous, although a few forms existed without this appearance. This finding confirmed our suspicion sufficiently so that intraperitoneal inoculation of macerated kidney tissue in saline was made into a guinea-pig. As may be the case, the animal did not develop a definite jaundice, but became somewhat indisposed. It was sacrificed one week after inoculation and the punctate pulmonary hemorrhages characteristic of this infection were demonstrated. From this lung macerated with sterile saline, large numbers of leptospiras with closely wound spirals were demonstrated by dark field technic.

Levaditi's stain on the patient's kidneys revealed definite silver positive forms with tapering ends found sparsely scattered throughout the degenerating kidney substance. These could be satisfactorily classified morphologically as leptospiras (fig. 1). Cultures were not secured because of the technical difficulties encountered in preparing a satisfactory medium as described by Noguchi, before the viable organisms were lost.

Having established the diagnosis, we were particularly anxious to determine the source of the infection. The relatives persistently refused to reveal the location of the property where the man had been working directly prior to his illness. This was described as an old carpet factory that had not been occupied for some years which was in untidy repair, with newspapers and rags lying about. Careful questioning of these relatives failed to reveal any other clue that might reasonably explain the infection. It is assumed therefore that he may have likely acquired the infection through the abraded or unbroken skin from contact with papers and rags contaminated with urine from infected rats. It is a simple matter

to accept the probable presence of rats in such a place as that described.

2. I. H., a mechanic, fifty-one years of age, entered the San Diego County General Hospital Jan. 16, 1932 presenting a deep jaundice. One month previously, at an unemployment camp, the patient was seized with chills and fever. His left knee became swollen and painful at this time, but subsided in three days. The chills and fever continued for two weeks and subsided just prior to the development of the jaundice. He was not seen by a physician during this time, so that the degree of fever is not known. He was not, however, confined to bed. As the jaundice developed and deepened he was seen by a physician who referred him into the hospital for diagnosis. At this time he complained of the jaundice with itching, black urine, and diarrhea with clay-colored stools. His temperature was normal and remained so until after operation one week later.

Until six months prior to admittance the patient had resided in Central America since 1910 and had malaria with yearly relapses since 1911, black water fever in 1924, and dysentery four times since 1928.

General examination at the time of admittance was negative save for the jaundice and some tympanites with abdominal tenderness. The spleen could not be palpated with certainty. The hemoglobin was 95 per cent (Sahli); erythrocytes, 4,680,000, and leukocytes, 9,000 per cu. mm. with 68 per cent neutrophils. The Kline and Wassermann tests were negative. Blood sugar was 100 mg. per cent. Icteric index 63; The Van den Bergh test gave a delayed biphasic reaction. Except for bile pigment, the urine showed no abnormality at this time. Three days later, the icteric index was 100. The next day his urine showed a distinct trace of albumin with many hyaline and granular casts and leukocytes. The stool was negative for bile on two occasions five days apart. X-rays of the gall bladder area revealed no calculi.

The patient was prepared for surgery by calcium administration. He was operated upon on January 27, 1932, eleven days after admission, with a diagnosis of obstructive jaundice, likely a calculus. The blood coagulation time was six minutes. No active process was discovered at operation that could account for the jaundice. A cholecystotomy was done and the wound closed in the usual manner. The operation was performed under spinal anesthesia. The post-operative condition was never good. The pulse was weak and thready and very rapid. The patient complained of pain in the lower abdomen and urine obtained by catheter was very bloody. He died on the second post-operative day.

An autopsy was performed eighteen hours after death, subsequent to embalming. The immediate cause of death was found to be a large hemorrhage, arising from the lower angle of the wound and extending into the prevesical space which it greatly distended. The blood also infiltrated the bladder at its base, explaining the bloody urine obtained during life.

No extra-hepatic cause for the jaundice could be discovered. The liver was normal in size but presented a marked degree of cloudy swelling and definite areas of focal necrosis observable grossly. The pancreas showed petechial hemorrhages. The spleen was moderately enlarged. The jejunum and upper ileum showed several hemorrhagic areas 2 cm. or more in diameter which were found on the mucosal side to be ulcerated. This undoubtedly had considerable to do with the diarrhea observed clinically. A horseshoe kidney was present which showed distinct degenerative changes of an acute type. Smears from the kidney stained by Fontana's method showed silver positive forms which were thought suggestive of leptospiras. Microscopically, the liver showed many focal areas of complete necrosis, sufficient to explain the clay colored stools on the basis of obstruction to bile excretion by the degenerating liver cells. The kidney also showed similar degeneration. Levaditi impregnation of the kidney revealed occasional silver positive spiral forms, in the degenerating kidney tubules (figs. 2 and 3). These were somewhat thicker in proportion to their length than those of case 1. They also presented fewer secondary spirals. In addition there were found large numbers of silver positive 'ring bodies' and some 'comma' forms suggesting a transition from one phase to the other. These were found in the tubules showing the most extensive degeneration of epithelium. Similar ring forms have been described in syphilitic tissues and are regarded by some^{16,17} as a pleomorphic form of the infecting agent.

Since the body had been embalmed prior to autopsy it was not possible to identify the organisms in any other way than by their morphology. A guinea pig was inoculated with a saline suspension of macerated kidney substance but the effort proved futile.

The history of this case is somewhat less complete than that of the first. The early symptoms were much less prostrating, but the progress of the case was of the same general type. Whether the years spent in Central America may in any way explain the decreased severity of the symptoms is problematical. It is even conceivable that a latent infection was acquired in Central America, but not activated until circumstances caused greater exposure. This is perhaps improbable.

Many organisms in the second case differ somewhat from those of the first. They appear more like spirillas than leptospiras. It is possible that the stage of the disease with the evidences of degenerated or involutional forms may account for the apparent decrease in the number of secondary spirals, or the organisms may be actually different types. The clinical symptoms and autopsy findings, however, warrant associating the two cases, at

least until such a time as a clearer differentiation can be made from the study of additional cases.

This second case occurred while the individual was living with an unemployment colony in the barracks of a former army training camp near San Diego. It is not known how the infection was acquired. No other cases have been recognized in the same camp.

A study of the human cases reported in North America makes it appear that this infection has been recognized only in cities of at least moderate size associated with some fairly large body of water. Whether this bears a relation to the incidence of rat carriers is unknown.

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THE LIGHT FILTERING ACTION OF BLOOD SERUM*

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It is a commonplace observation that human blood serums differ in their color and relative opacity to light. While these variations have been studied from the chemical standpoint, so far as I have been able to ascertain, no study has been made of the light filtering action of human blood serum. Our original investigations were based upon photographic procedures which were found to be too complicated for clinical purposes and were therefore discarded. A series of subsequent investigations using the Exton scopometer were also discarded since that instrument was not suited for the type of investigation undertaken. The results recorded in this paper were obtained with the Pulfrich photometer.

METHOD

This photometer consists of a lamp housing, in front of which is a prism which splits the light into two parallel beams of equal intensity. In front of these light beams are placed two standardized cells, which for the purposes of the present investigation have a thickness between the upright walls of 2.5 mm. In the one cell distilled water is placed, while the serum under observation is placed in the other. Next comes an observation tube containing a prism which brings the light beams in contact so that each beam illuminates one half a circle. Between the eyepiece and this prism is a holder in which light filters may be placed. In the present study filters having as their center of filtration the following wave lengths were employed; 7200 Å, 5700 Å, 5000 Å, 4300 Å. In subsequent discussion these filters will be referred to by the first two figures only the zeros being dropped. The filters are so standardized that while they cover a band approximately 100 Å units each side of the designated wave length, their center has, by spectroscopic examination, been determined to be at the point indicated. On each side of the instrument is a wheel which, when rotated, closes a square iris gradually cutting off the light. This wheel is graduated on

* Under a grant from the Fred. Weingarten Fund.

its circumference in percentages, so arranged that the percentage indicated is the amount of light transmitted although the manipulation of the wheel actually cuts off light. In actual use the wheel on the side containing the distilled water is rotated until both fields of light are of equal intensity. The more exact details of the technical procedure are given in the booklet furnished by the manufacturers.

Blood was collected in the usual fashion, by venepuncture, under the standard conditions of procedure as for a blood chemical examination, before breakfast, or after a fast of approximately twelve hours. The serum was rapidly

TABLE 1
LIGHT FILTRATION OF VARIOUS SERUMS

| DIAGNOSIS | TYPE A | | | | | TYPE B | | | | | TYPE C | | | | |
|--------------------------------------|---------------------|----------|--|-------|-------|---------------------|----------|--|-------|-------|---------------------|----------|--|-------|-------|
| | Number of specimens | Per cent | Averaged rates; light filtration for each band | | | Number of specimens | Per cent | Averaged rates; light filtration for each band | | | Number of specimens | Per cent | Averaged rates; light filtration for each band | | |
| | | | 72-57 | 57-50 | 50-43 | | | 72-57 | 57-50 | 50-43 | | | 72-57 | 57-50 | 50-43 |
| Normals..... | 42 | 40 | 0.6 | 2.3 | 3.3 | 19 | 20 | 1.9 | 1.4 | 5.1 | 43 | 40 | 0.5 | 4.3 | 2.4 |
| Arterio-sclerosis, hypertension..... | 24 | 57 | 0.6 | 2.4 | 3.9 | 5 | 12 | 1.5 | 1.4 | 5.0 | 13 | 31 | 0.3 | 3.6 | 2.4 |
| Mental disturbance..... | 5 | 28 | 1.0 | 2.6 | 4.2 | 2 | 11 | 1.9 | 1.4 | 5.3 | 10 | 61 | 0.6 | 5.4 | 4.8 |
| Endocrine dysfunction.... | 8 | 40 | 0.5 | 2.2 | 3.2 | 6 | 30 | 1.8 | 1.3 | 5.6 | 6 | 30 | 1.1 | 4.0 | 2.0 |
| Chronic bacterial infection..... | 17 | 34 | 0.6 | 2.0 | 3.3 | 5 | 11 | 2.4 | 1.2 | 5.0 | 27 | 55 | 0.6 | 3.0 | 2.0 |
| Acute bacterial infection.. | 33 | 37 | 0.6 | 2.0 | 3.3 | 9 | 10 | 1.8 | 1.1 | 4.9 | 46 | 53 | 0.4 | 3.0 | 1.9 |
| Syphilis..... | 20 | 36 | 0.5 | 1.9 | 3.7 | 13 | 24 | 1.7 | 1.4 | 4.9 | 22 | 40 | 0.7 | 3.2 | 2.4 |
| Fractures..... | 7 | 35 | 0.7 | 2.1 | 3.6 | 4 | 20 | 2.0 | 1.3 | 5.3 | 9 | 45 | 0.3 | 3.0 | 2.9 |
| Pregnancy..... | 10 | 58 | 0.6 | 2.1 | 3.4 | 3 | 19 | 3.3 | 1.3 | 5.6 | 4 | 23 | 0.5 | 2.3 | 1.7 |
| Healing wounds..... | 9 | 50 | 0.7 | 2.4 | 4.2 | 2 | 11 | 4.7 | 0.8 | 5.4 | 7 | 39 | 0.9 | 2.8 | 2.3 |
| Benign tumors..... | 3 | 13 | 0.6 | 2.0 | 3.9 | 9 | 39 | 2.3 | 1.3 | 4.9 | 11 | 48 | 0.6 | 3.3 | 2.9 |
| Malignant tumors..... | 7 | 30 | 0.5 | 1.7 | 3.9 | 5 | 21 | 1.6 | 1.0 | 5.7 | 11 | 49 | 0.5 | 3.4 | 1.7 |

separated from the clot. Hemolized serums were discarded, and the examination was made within twelve hours after the collection of the specimen.

The serum having been separated, one cell was filled with it, the other with distilled water. In the manner indicated in the booklet of instructions, the transmission of light for each of the wave lengths was determined. The rate of absorption of light per 100 Å units for each band between the filters was then determined, as in the following hypothetical example.

Readings; 72 Å, 78 per cent; 57 Å, 70 per cent; 50 Å, 51 per cent; 43 Å, 35 per cent. There are 1500 Å units between 57 and 72, and 700 Å units in each of the other two bands. The band 57 to 72 filtered out 8 per cent of the light

(78 per cent minus 70 per cent). The band 50 to 57 filtered out 19 per cent, and the band 43 to 50 filtered out 16 per cent. The rate in the first band was 8 divided by 15 or one half of one per cent, in the second band 19 divided by 7 or 2.7 per cent and in the third band 16 divided by 7 or 2.2 per cent.

If the three percentages thus obtained are presented in graphic form it will be found that three types of curve occur (see fig. 1). In one termed A, the rate

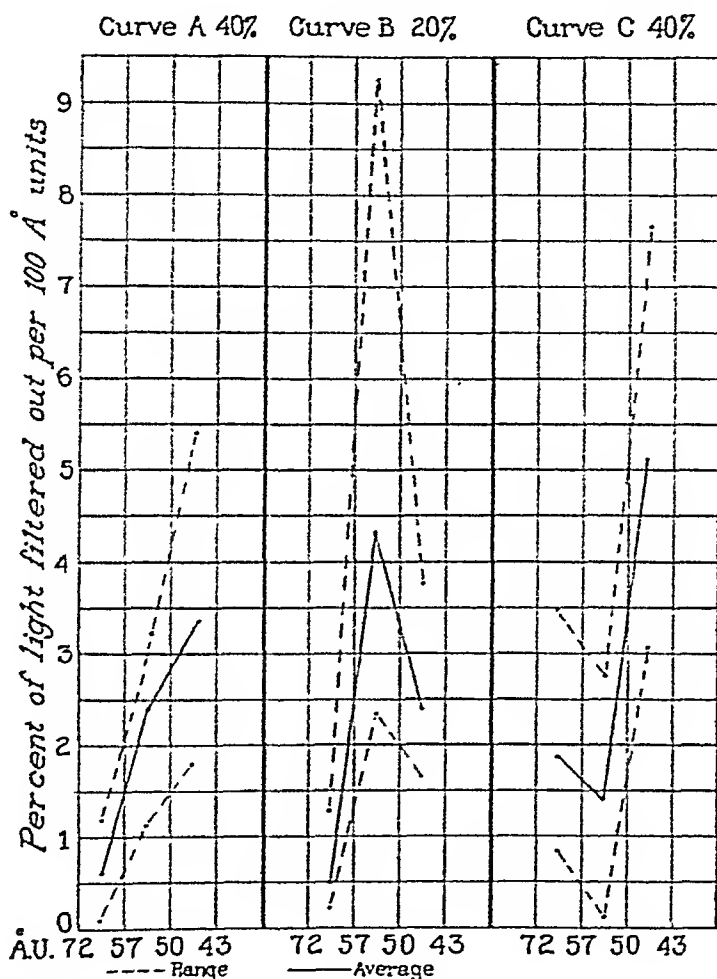


FIG. 1. TYPICAL CURVES

of filtration increases progressively. In the second type termed B, the maximum rate of filtration occurs in the middle band. In the third type, termed C, the minimum rate of filtration occurs in the middle band.

An analysis of 104 normal serums made on the classification just described demonstrates the following: the range of the

reaction, when different serums are compared, is wide (see broken lines fig. 1), the curve types are divided as follows A, 40 per cent, B, 20 per cent, C, 40 per cent. An analysis of the phenomena in the serums of 372 cases suffering from a variety of pathological conditions will be considered in a later paragraph.

There are a number of factors which influence the rate of filtration. There is an increasing rate of filtration with increasing age. This is most manifest in the band 43 to 50. In this band under twenty years of age the averaged rate is 3.6 per cent, from 20 to 40 years it is 4.1 per cent, from 40 to 60 years it is 4.6 per cent and over 60 years it is 4.7 per cent per 100 Å units. In general the serums from males filter out slightly more light than those from females, thus in type A the rate for females is 3.9 per cent; for males 4.6; in type B the rate for females is 2.3 per cent; males, 2.5; while in type C the rate for females is 4.8 per cent; males, 5.8.

Increasing the elapsed time between taking the specimen and examining it, may alter the type of reaction, and increases the rate of filtration. As illustrative, a given serum when fresh had an A curve with an averaged filtration rate per 100 Å units of 0.5 per cent; twenty-four hours later the reaction type was A and the averaged rate of filtration 1.4 per cent; forty-eight hours after taking the specimen the reaction was of the B type and the filtration rate was 1.5 per cent.

Radiation of the serum with ultraviolet light, one skin erythema dose being given and the serum being cooled with a fan, increased the filtering power of the serum and occasionally changed the type of reaction.

Inactivation of complement, as is done in the test for complement fixation, often changed the type of reaction and either increases or decreases the filtering power. Further investigation showed that those serums containing natural amboceptor had an increased filtering power after inactivation, while in those serums without natural amboceptor the filtering power decreased after inactivation. No mathematical correlation could be established between the amount of natural amboceptor present and the degree of increase in the filtration rate. As typical of

the change two instances are cited. In one serum without natural amboceptor the initial filtering rate was 2.7 per cent, which after inactivation fell to 2.3 per cent. In another specimen containing natural amboceptor the initial rate was 2.6 per cent which after inactivation rose to 3.1 per cent.

The constancy of the filtration rate in the same individual was studied in ten cases, specimens being taken at from four to thirty-five day intervals. It was found that in those individuals in whom the clinical condition remained unchanged the filtration rate was constant, thus in a case of chronic productive arthritis the initial rate was 3.7 per cent seven days later it was 3.8 per cent; a case of neurasthenia had an initial rate of 2.8 per cent and twenty-two days later 2.7; a case of senile dementia had an initial rate of 2.1 per cent and four days later the same rate. In none of these patients had there been any decisive change in the clinical condition during the period of observation. In contrast, patients in whom there had been a decided change in the clinical condition showed marked changes in the filtration rate. In a case of acute appendicitis the initial rate was 1.7 per cent, fourteen days later when clinically improved, 2.2 per cent. In a case of fibroid of the uterus, the initial rate was 3.7 per cent, twelve days later (three days post-operative), 1.9 per cent. In a case of inguinal hernia, the initial rate was 3.1 per cent, eleven days later (five days post-operative) 1.3 per cent. While this phenomena will be the subject of a later communication describing its application in prognosis it may be summarized here that as the clinical condition improves the rate rises, and as the clinical condition gets worse the rate falls.

With five serums the total protein content of each was determined directly after the readings, while with five others the readings were followed by determination of the lipid content. No relationship between the amount of light filtered out or the reaction type and either the protein or lipid content could be demonstrated.

In other observations it was noted that if definite amounts of either normal acid, or alkali, were added to definite amounts of serum that there were changes both in the type of reaction and in

the degree of filtration. In general the addition of either acid or alkali decreased the rate of filtration, the decrease in the filtration rate averaging four-tenths of one per cent.

A statistical summary of the light filtering power of serum in 372 cases of various disease conditions is presented in table 1. The wide range of the reaction when individual serums were compared rendered it impossible to demonstrate consistent variations between individual serums, but when groups were averaged certain trends of reaction became apparent. It is believed that group averaging is permissible since light filtration is a physico-biological process in which for the present we are interested in determining trends rather than estimating the mathematical degree of change.

The various conditions studied have been rather broadly grouped and individual diagnoses are not given. It was noted that the percentage of each group in the various types of reaction varied. Thus 40 per cent of the normal individuals gave a type A curve while in the pathological serums the lowest rate of type A curve was in the benign tumors which gave 13 per cent, and the highest rate was in pregnancy which gave 58 per cent. Normal serums showed 20 per cent to give type B curve while with pathological serums the lowest rate was given by acute bacterial infections (10 per cent), the highest rate (39 per cent) being given by benign tumors. With the type C curve the normals gave 40 per cent while the lowest rate of the pathological sera (30 per cent) was found in a group of endocrine dysfunctions, the highest rate (61 per cent) occurred in a group of mental cases.

If the filtration rate in each reaction type and for each diseased group be compared with the normal, variations are again observed but such variations occur without any definite rule. In the type A reaction all of the group averages with the exception of endocrine dysfunctions, and acute and chronic bacterial infections, showed a higher filtration rate than the normals. With the type B reaction, all the groups except the mental cases showed lower filtration rates than the normals. With type C curve a still different arrangement of the groups occurred.

It is evident therefore that no diagnostic significance can be attached to a determination of the light filtering power of serum, although as has been previously suggested there is apparently a definite aid in prognosis associated with its determination.

SUMMARY

It is apparent that human blood serum has the power to filter out light in the range 7200 to 4300 Å. This filtering action varies with different serum both in intensity and point of maximum filtering power. The various factors which affect the filtering power, namely, age, sex, heat, age of specimen, ultraviolet radiation, and disease states, plus the observation that protein and lipoidal content of the sera are not in relation to the filtering power, suggests that the filtering power is dependent upon the colloidal state of the serum. The nature of the changes in the colloidal state can at the present be only a matter of theory since experimental observations are lacking.

I beg to express my appreciation to Miss Miriam Berke for technical assistance.

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PERMANENT COLOR STANDARDS FOR FOLIN'S FERRICYANIDE SUGAR METHOD

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Folin's^{1,2} ferricyanide method for blood sugar estimation has been found admirably applicable to the determination of sugar in spinal fluids. This method has the distinct advantage of requiring only 0.1 cc. of spinal fluid, whereas the Folin-Wu copper method requires 2 cc. In order to make this ferricyanide method rapidly and intermittently available for general clinical and hospital practice in the estimation of spinal fluid sugar, it seemed desirable to devise a permanent standard whereby the unstable sugar standard and the colorimeter could be eliminated. Standards so prepared furnish a rapid means for sugar measurement in spinal fluids comparable to the accepted colorimetric procedures for icterus index and the phenolsulphonephthalein tests.

Folin's method is essentially the reduction of an alkaline solution of potassium ferricyanide by the sugar and the precipitation of the resulting potassium ferrocyanide as Prussian blue by the addition of ferric iron. The precipitated Prussian blue is kept in a colloidal suspension by the use of gum ghatti. Sugar values vary with the depth of the blue color produced.

METHOD OF PROCEDURE

The technique as used by Folin and Malmros³ was followed with the exception that 1 cc. of a 0.2 per cent solution of potassium ferricyanide, as originally recommended, was used rather than 2 cc. of a 0.4 per cent solution because a better range of color is obtained in the lower end of the scale with the use of the more dilute ferricyanide solution. This is especially desirable when the method is applied to spinal fluids since the amount of sugar found in normal spinal fluid is relatively low, from 50 to 70 mgm. per cent.

Reagents

1. Dilute Tungstic Acid Solution.

Transfer 20 cc. of a 10 per cent sodium tungstate solution to a liter volumetric flask. Dilute to a volume of about 800 cc., add 20 cc. of $2/3$ N sulphuric acid and then dilute the whole to one liter.

2. Potassium Ferricyanide Solution, 0.2 per cent.

Dissolve 1 gram of potassium ferricyanide C.P. in distilled water and dilute to 500 cc.

3. Sodium Cyanide-Carbonate Solution.

Dissolve 8 grams of anhydrous sodium carbonate in 50 cc. of water. Add 150 cc. of a 1 per cent solution of freshly prepared sodium cyanide solution. Mix and dilute to 500 cc.

4. Ferric Iron Solution.

Fill a liter cylinder with distilled water. Suspend on a copper wire screen, just below the surface of the water, 20 grams of soluble gum ghatti* and allow to stand over night. Remove the screen and strain the liquid through a double layer of cheese cloth. Dissolve 5 grams of anhydrous ferric sulfate in a mixture consisting of 75 cc. of 85 per cent phosphoric acid and 100 cc. of distilled water and add this solution to the solution of gum ghatti. Add sufficient 1 per cent potassium permanganate solution to destroy the reducing materials present in gum ghatti so that a slight pink color will persist for about five minutes (between 15 and 40 cc. will be required). Care should be taken that there is no pink color present in the reagent when used.

5. Stock Glucose Solution.

Dissolve 1 gram of benzoic acid in 200 to 300 cc. of water. Add exactly 1 gram of glucose C.P. and dilute to 500 cc. One cubic centimeter of this solution contains 2 mgm. of glucose.

6. Standard Glucose Solution.

Dissolve 0.25 gram of benzoic acid in 500 to 600 cc. of water, add 5 cc. of the stock solution of glucose (reagent 5) and dilute to 1 liter. One cubic centimeter of this solution contains 0.01 mgm. of glucose. (These sugar solutions will not be needed unless it is desired to check the permanent color standards.)

Technique of the Determination

Pipette 10 cc. of the dilute tungstic acid solution into a centrifuge tube. Add exactly, from a 0.1 cc. capillary pipette, 0.1 cc. of spinal fluid (or blood) to the tungstic acid solution. Care should be taken to first wipe off the outside of the pipette with a piece of filter paper so that exactly 0.1 cc. is measured. Mix well and then centrifuge for about five minutes. Measure exactly 4 cc. of the supernatant fluid into a Pyrex tube. Add 1 cc. of the 0.2 per cent solution of potas-

* Soluble gum ghatti may be obtained from Arthur H. Thomas Co., Philadelphia or Eimer and Amend, New York City.

sium ferricyanide and 1 cc. of the cyanide-carbonate solution. Heat in a boiling water bath for exactly eight minutes. Cool immediately in a cold water bath for about two minutes, add 5 cc. of the ferric iron solution, mix and let stand for five minutes. Add sufficient water to bring the total volume of the mixture to 25 cc. and compare with the permanent color standards directly.

In the case of pathological conditions such as diabetes mellitus where the sugar content is high it is only necessary to dilute the color formed with a diluting solution prepared from the stock reagents. If the color is above the highest standard, remove an aliquot part of the prepared colored sample and dilute to 25 cc. with a freshly prepared diluting solution made by adding 4.0 cc. of 0.2

TABLE 1
PREPARATION OF PERMANENT COLOR STANDARDS*

| SUGAR** | 20 PER CENT COPPER SULPHATE $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ | 5 PER CENT CHROME ALUM $\text{Cr}_2(\text{SO}_4)_3 \cdot$ $(\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$ | 0.5 PER CENT GOLD CHLORIDE $\text{AuCl}_3 \cdot 2\text{H}_2\text{O}$ | 2.4 PER CENT COBALTOUS CHLORIDE $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ |
|----------------------|---|--|--|--|
| <i>mgm. per cent</i> | <i>cc.</i> | <i>cc.</i> | <i>cc.</i> | <i>cc.</i> |
| 0 | 0 | 0.2 | 2.3 | 0 |
| 10 | 0.1 | 0.2 | 2.0 | 0 |
| 20 | 0.5 | 0.4 | 1.8 | 0 |
| 30 | 0.9 | 0.6 | 1.3 | 0 |
| 40 | 1.5 | 1.0 | 1.0 | 0 |
| 50 | 2.1 | 1.7 | 0.6 | 0 |
| 60 | 2.8 | 2.0 | 0.3 | 0 |
| 70 | 4.2 | 2.3 | 0 | 0.7 |
| 80 | 6.8 | 2.1 | 0 | 2.5 |
| 90 | 9.5 | 2.0 | 0 | 3.5 |
| 100 | 12.0 | 2.0 | 0 | 4.0 |

* Each tube contains 2 drops of concentrated HCl and the total volume is made to 25 cc.

** Standards read in milligrams per cent if 0.1 cc. of spinal fluid is used and if 4 cc. of the clear supernatant centrifugate is taken.

per cent ferricyanide solution to 20 cc. of gum ghatti iron solution and diluting to 100 cc. By utilizing a suitable aliquot of the final solution it is possible to measure as much as 200 mgm. per cent of sugar. If more than this amount is present it becomes necessary to use a smaller aliquot of the tungstic acid centrifugate diluted to 4 cc. in order to keep the sugar concentration within the oxidizing limits of the potassium ferricyanide present. By the use of these dilution methods it is possible to measure sugar values from 10 to 500 mgm. per cent with the use of the permanent color standards.

Experimental preparation of standards

Known quantities of a freshly prepared standard sugar solution (1 cc. containing 0.01 mgm. of sugar) were used to set up a series of tubes representing

from 0 to 100 mgm. per cent of sugar when 0.1 cc. sample of spinal fluid is taken. Efforts were then made to match these colors with synthetic solutions consisting of varying amounts of copper sulfate, potassium dichromate, chrome alum, and cobaltous chloride. It was found possible by a cut and try method to match these colors with mixtures of these solutions. However, after some length of time, it was observed that in the lower part of the range the yellow tint had diminished. Substitution of a gold chloride solution for potassium dichromate was found to be satisfactory. Two drops of concentrated hydrochloric acid were added to each tube to prevent the separation of metallic gold and the tubes then sealed to prevent evaporation. These standards have kept for four months. Table 1 gives the amounts of each of the four solutions needed to make up the standards.

Pyrex test tubes may be used for running the determination. For comparison the solutions should be transferred to clear glass tubes of the same diameter as those in which the standards are kept. In this laboratory 25 cc. clear glass cylindrical ampules 2.25 cm. in diameter were used. The ampules were viewed from the side against a white background. It is possible to interpolate values between the standards. Differences of 5 mgm. per cent can be very easily seen.

PERMANENT COLOR STANDARD AND COLORIMETER READINGS COMPARED

Comparisons of the amount of sugar found in spinal fluids were made on a number of samples first by matching with the permanent color standards and then by comparison with a known sugar standard representing 50 mgm. per cent in a colorimeter. A 50 instead of a 100 standard was used since it is nearer the range of the sugar content of normal spinal fluids.

The sugar values on thirty-eight spinal fluids were determined by these two methods. Considering the colorimeter readings as valid, the permanent standard results on twenty of these fluids showed a variation of ± 1.0 mgm. per cent. Eleven samples gave results varying between ± 1.0 and ± 2.0 mgm. per cent and six of them gave slightly greater variations. In only one case the value obtained with the use of the permanent standards was 4.0 mgm. per cent higher than that obtained by the colorimeter.

In another set of experiments sugar determinations were made by the Folin-Wu copper molybdate method⁴ at the same time as the Folin ferricyanide method on fifteen different samples. A variation between the two methods was found amounting for the most part to a difference ranging from 0.8 to 3.4 mgm. per cent.

In three samples differences of 4.4, 5.3, and 5.4 mgm. per cent were found. In ten out of the fifteen samples the values found with the permanent standards were between the results obtained by the two colorimeter methods. Results show that the amount of sugar obtained with the Folin-Wu copper method and the amount obtained with the Folin ferricyanide method, either with the use of the permanent color standards or the colorimeter, does not vary greatly.

It is believed that the use of permanent color standards is feasible in the determination of sugar by the Folin ferricyanide method. It eliminates the undesirable features incident to the use of a sugar standard and also the expense involved in procuring a colorimeter. While the results obtained by simple visual comparison of color are not as accurate as those obtained by mechanical measurement, the results in either case are more accurate than necessary from the standpoint of clinical interpretation. The ease and rapidity with which a determination is made by the use of permanent color standards should commend their use to clinical practice.

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A COMPARISON OF THE RELATIVE VALUES OF THE INTRACUTANEOUS SKIN TEST AND OF THE PATHOGEN-SELECTIVE CULTURE IN SELECTING BACTERIA FOR VACCINES FROM MIXED CULTURES*

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In discussing vaccines, Kolmer and Boerner² enumerated eleven general principles of which the last two are as follows:

10. The pathogen-selective^{1,3} method (given below) may be helpful in selecting bacteria for vaccines in mixed infections on the basis that only those capable of growing in the whole coagulated blood of the patient are apt to be virulent. It is possible, however, that an organism unable to grow in the blood or produce septicemia may still be able to produce local infection.

11. Intracutaneous skin tests are also advocated for the selection of bacteria in the preparation of vaccines on the principle that only those yielding positive reactions should be employed. Positive skin reactions may be an indication of acquired allergic sensitization and therefore evidence of infection with the organism; they may be also plain inflammatory reactions if the inoculum contains a toxin or toxins for which there are no or insufficient amounts of antitoxin in the blood. The exact value or status of the method is as yet unknown.

In mixed cultures, in order to test the value of intracutaneous tests as a means of differentiating those organisms that are capable of infecting the patient from those against which he possesses good resistance, the reactions produced by the intracutaneous injections of those organisms that grew in the patient's fresh, whole, coagulable blood were compared with the reactions produced by those organisms that were killed by this blood.

METHOD

The patients cultured and tested were in the pediatric ward of the Mt. Sinai Hospital, Philadelphia. The bacteriological work was done in the hospital laboratory by Miss Marian Barnes.

* Read at a joint meeting of the American Society of Bacteriologists and Pathologists and the American Association of Immunologists, April 28, 1932.

Twenty children were thus studied, and also eight adults for whom pathogen-selective vaccines containing but a single organism were made by Dr. Fred Boerner, in the Laboratory of the Graduate Hospital of the University of Pennsylvania.

A swab containing the inoculum first was rubbed on the bottom of a sterile test-tube, into which one or two drops of broth had been placed, after which the swab was inserted in a tube of hormone broth medium. About 5 cc. of the patient's fresh, whole, coagulable blood were then placed in the first inoculated tube. After twenty-four hours incubation the tubes were subcultured on blood-agar. Each organism present in the subcultures was then grown in pure culture in a hormone broth medium. After four days growth in the incubator enough 5 per cent solution of tricoresol was added to the pure culture of each organism to make 0.5 per cent of the total volume. The following day each vaccine was cultured for sterility and if found sterile was placed in a small ampule. The ampules therefore contained all the organisms that grew in the patient's blood owing to the lack of bactericidal power against them in the blood, and also all those organisms that failed to grow in the patient's blood, because of the presence in it of bactericidal power against them. The exogenous toxins produced by the contained organism also were in each ampule. The vaccine in each ampule was injected intracutaneously in the forearm of the patient from whom the organisms were obtained.

Eighty-four organisms were studied, sixty-three that grew in the patient's blood and twenty-one that failed to grow in the patient's blood.

In studying the reactions the areola and induration were charted separately, inasmuch as there seemed to be little correspondence between them, as will be seen from the following summary:

The areola and induration was of the same degree in 32 per cent of tests with organisms that grew in the patient's blood and 9 per cent with those that failed to grow in the blood; the areola was more marked than the induration in 49 per cent of the tests with organisms growing in the patient's blood and 86 per cent of those not growing; the induration was more marked than the areola in 19 per cent of the tests with the organisms growing in the patient's blood and 5 per cent of those not so growing. Thus the induration was of the same degree or of greater intensity in 41 per cent of tests with organisms growing in the patient's blood and 14 per cent of those not growing in the same blood.

A comparison of the areolas produced by intracutaneous injection of organisms that grew and of those that failed to grow in the patient's blood is given in table 1. No difference between the two groups was noted in areolas of slight, moderate or marked intensity. A larger percentage of the still stronger reactions, however, was produced by the organisms that failed to grow in the blood, but in no instance did the latter produce a very mild or no areola, although these results occurred in 13 per cent of the injections of organisms that grew in the patient's blood.

TABLE 1

COMPARISON OF DEGREES OF AREOLAS AND INDURATED AREAS IN INTRACUTANEOUS TESTS WITH VARIOUS ORGANISMS

| DEGREE OF REACTION | AREOLAS | | | | INDURATED AREAS | | | |
|--------------------|-------------------------------------|----------|---|----------|-------------------------------------|----------|---|----------|
| | Bacteria growing in patient's blood | | Bacteria not growing in patient's blood | | Bacteria growing in patient's blood | | Bacteria not growing in patient's blood | |
| | num-ber | per-cent | num-ber | per-cent | num-ber | per-cent | num-ber | per-cent |
| - | 6 | 10 | | | 10 | 16 | 1 | 5 |
| ± | 2 | 3 | | | 1 | 2 | | |
| + | 10 | 16 | 2 | 9.5 | 16 | 25 | 7 | 33 |
| ++ | 14 | 22 | 4 | 19.0 | 26 | 41 | 11 | 52 |
| +++ | 15 | 24 | 7 | 33.0 | 7 | 11 | 2 | 10 |
| ++++ | 11 | 17 | 5 | 24.0 | 3 | 5 | | |
| +++++ | 5 | 8 | 2 | 9.5 | | | | |
| ++++++ | | | 1 | 5.0 | | | | |
| - and ± | 8 | 13 | | | 11 | 18 | 1 | 5 |
| + to +++ | 39 | 62 | 13 | 61.5 | 49 | 77 | 20 | 95 |
| ++++ to ++++++ | 16 | 25 | 8 | 38.5 | 3 | 5 | | |

The intensity of the induration produced by the two groups is contrasted in table 1. Little difference is shown except that a greater per cent showing no induration, followed the injection of organisms that grew in the patient's blood.

In table 2 the different organisms are indicated with the various types of reaction they produced, those that grew in the patient's blood being recorded separately from those that were killed by the blood.

The amount of time required for the reaction to reach its maximum intensity was studied to see if there was any difference

between the two groups, but none was found. Approximately the same per cent of reactions reached their maximum intensity both for the areola and the induration, whether the organisms tested grew in the patient's blood or not, either within a day or within two or more days. About twice the per cent of areolas

TABLE 2

| ORGANISMS | AREOLAS | | | | | | | | INDURATED AREAS | | | | | | | | | | | | |
|--|-------------------------------------|----|----|----|---|--------|----|----|-------------------------------------|----------|--------|----|---|----|----------|--------|----|----|----|----------|---|
| | Bacteria growing in patient's blood | | | | Bacteria not growing in patient's blood | | | | Bacteria growing in patient's blood | | | | Bacteria not growing in patient's blood | | | | | | | | |
| | - or # | 1+ | 2+ | 3+ | 4+ to 6+ | - or # | 1+ | 2+ | 3+ | 4+ to 6+ | - or # | 1+ | 2+ | 3+ | 4+ to 6+ | - or # | 1+ | 2+ | 3+ | 4+ to 6+ | |
| <i>Streptococcus</i> (green producing)..... | | | 2 | 1 | | | 2 | 1 | 3 | | | 2 | 1 | | | | | 3 | 3 | | |
| <i>Streptococcus</i> (hemolytic)... | 1 | 2 | | | | | | 1 | 2 | | | 1 | 2 | | | | | 1 | 2 | | |
| <i>Streptococcus</i> (sl. hemolytic)... | | | | | | | | 1 | | | | | | | | | | 1 | | | |
| <i>Streptococcus anhemolyticus</i> ... | 2 | | 2 | 4 | | | 1 | 2 | | 2 | 1 | 2 | 5 | | | | 1 | 1 | 3 | | |
| <i>Streptococcus</i> (anaerobic)... | | | | | | | 1 | | | | | | | | | | | 1 | | | |
| <i>Staphylococcus aureus</i> | 1 | 1 | 1 | 1 | 2 | | 2 | 1 | 4 | 6 | 1 | 1 | 3 | 1 | | | 1 | 2 | 8 | 1 | 1 |
| <i>Staphylococcus albus</i> | 3 | | 2 | | 5 | | | | 3 | 2 | 1 | 3 | 4 | 2 | | | 2 | 2 | | | 1 |
| <i>Pneumococcus</i> | | | 1 | 1 | 1 | | | 2 | 1 | 1 | 1 | 2 | | | | | | 1 | | 2 | 1 |
| <i>Gaffkya</i> (<i>Micrococcus</i>) <i>tetragena</i> | | | | | | | | | 1 | | | | | | | | | | 1 | | |
| <i>Neisseria sicca</i> | | | | | | | 1 | | | 1 | | | | | | | 1 | | 1 | | |
| <i>Neisseria flava</i> | | 1 | | | | | | | | | | 1 | | | | | | | | | |
| <i>Escherichia coli</i> (<i>B. coli</i>).... | | | | | 1 | | | 1 | | 1 | 1 | | | | | | 1 | | 1 | | |
| <i>Pseudomonas aeruginosa</i> (<i>B. pyocyanus</i>)..... | | | | | | | | 1 | | | | | | | | | | | 1 | | |
| <i>Klebsiella pneumoniae</i> (Friedlander's bacillus)..... | | | | | | | 1 | | | | | | | | | | 1 | | | | |
| <i>Hemophilus influenzae</i> | | | | | 1 | | | | | | | | 1 | | | | | | | | |
| <i>Diphtheroids</i> | | | | | | | | 1 | | | | | | | | | | 1 | | | |
| Totals..... | 7 | 4 | 8 | 7 | 10 | 1 | 7 | 11 | 14 | 13 | 5 | 12 | 15 | 4 | | | 7 | 11 | 20 | 5 | 3 |

reached their maximum degree of reaction at the end of one day as those that became maximal at two days or more. Of those showing induration, about 50 per cent were maximal within twenty-four hours.

The duration of the reaction was also studied. The results showed there was practically no difference in the duration of the

reactions produced by those organisms growing and those failing to grow in the patient's blood. In both groups approximately half of the areolas persisted for three days or less, the rest lasting five days or more. Slightly more than half of the indurated areas resolved within three days, while the remaining ones lasted five days or more.

CONCLUSIONS

(1) There is practically no difference between the reactions produced by intracutaneous injections of organisms that are able to grow in the patient's fresh, whole, coagulable blood and the reactions produced by organisms that are killed by his blood.

(2) There seems to be no correspondence between absence of bactericidal power in the blood of the host against a given organism and production in that host of a positive reaction by the intracutaneous injection of such organism.

(3) There probably is no relationship between hypersensitive-ness in the host to the exogenous and endogenous toxins of a given organism and the pathogenicity of such organism for that host.

(4) It is questionable whether intracutaneous tests can identify, in a mixed culture, the bacteria that are infecting the patient.

(5) Therefore, intracutaneous skin tests are probably unreliable for selecting bacteria in the preparation of vaccines.

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THE HISTOPATHOLOGY OF METHYL ALCOHOL POISONING*

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One of the earliest references to the toxicity of wood alcohol is in an article published by MacFarlan¹⁴ between the years 1855 and 1856 in London. Approximately twenty-five years later a French investigator, Poincaré,¹⁸ carried on experimental work upon the toxic effect of its vapors. From that time to the present literature contains many articles concerning the toxicity of this substance. As early as 1880 it was found to be toxic regardless of its portal of entry and also that the toxic manifestations were widespread throughout the body. Even Poincaré mentioned such changes in the liver, heart, kidneys, and lungs of his experimental animals. Holden⁹ observed degenerations of the ganglion cell layer of the retina (with Nissl stain), and degenerations of the medullary sheath affecting some fibers of the optic nerve. These changes were accompanied by numerous hemorrhages into the meninges of the cord and brain.

Following the development of the ophthalmoscope, clinical observation of the eye grounds became more common, and the changes in this organ grew in importance until methyl alcohol became generally considered as a specific toxin for the retina and in particular for the optic nerve. This is exemplified in the statement of Wood and LaWall,²⁷ "that this predilection for the optic nerve is but a part of the general tendency toward nerve trunks," and that of Tyson,²³ who stated that while these toxic manifestations are most pronounced in the retina and optic nerve, possibly other cranial nerves are also affected.

The tissues forming the basis of the present study resulted from

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a series of experiments made by Dr. Carey P. McCord¹⁵ in the Industrial Health Conservancy Laboratories of Cincinnati, Ohio, in which a careful study of the toxic effects of methyl alcohol following various methods of administration was made. A very brief summary of that work is as follows.

The animals used were thirty-one young rhesus monkeys, fifty-eight rabbits of four breeds, and 176 white rats. Of these the final number having tissues available for microscopic study were forty-one monkeys, twenty-six rabbits, and eighty-one rats. These animals were treated by skin absorption, inhalation, and ingestion. The materials used were various grades of natural and synthetic methyl alcohol, samples of which had been obtained both from the manufacturer and upon the open market. Details concerning the manner of application can be found in Dr. McCord's discussion.

PRESENT STUDIES

No animal was utilized for tissue study unless found before the body warmth had left the corpse. Autopsies were performed immediately and portions of tissue preserved in the proper fixatives. The remainder of the organs was tested chemically for the presence of methyl alcohol or its decomposition products.

The eyes of all monkeys and all rabbits except albinos were examined at least once by a competent ophthalmologist.

Intoxication was evident following all methods of application. The first sign of intoxication was a dilatation of the pupils, which reacted slowly to light. This was followed by an illness which sometimes led to prostration and possibly to death. Recovery from the effects of methyl alcohol frequently took place even during sustained exposure. Animals which had been blind upon ophthalmoscopic examination frequently regained sight and a subsequent examination revealed no pathology in the eye grounds. The brand or purity of the alcohol seemed in no way to affect the pathological changes. Animals subjected to methyl alcohol have regularly shown the presence of this substance in the blood, urine, and tissues. Formaldehyde was never recovered in any appreciable amounts.

By inhalation the threshold of danger is well below 1000 p.p.m. of methyl alcohol vapor, while by skin absorption the threshold is near 0.5 cc. per kilogram of body weight applied four times daily. Individual animals vary greatly in susceptibility, black animals being extremely resistant. There is some species variation in that rats are most susceptible and rabbits most resistant.

The typical histopathological picture of the tissues of these animals following methyl alcohol poisoning varied more with the species of animal used than upon the method of administration. Upon repeated contacts of the skin to alcohol it showed evidence of irritation, which sometimes changed to actual ulceration and necrosis. Irritation of the skin was noted by Rosenau²⁰ in individuals poisoned by methyl alcohol. Dublin and Lieboff⁴ mentioned inflammation of the throat and mucous membranes of the air passages among the clinical manifestations of the poisoning following inhalation of methyl alcohol fumes.

Following either method of administration the organs of the abdomen most constantly affected were liver, spleen, and kidneys. The reaction in the liver was practically always one of parenchymatous degeneration, which, in the more severe cases, had proceeded to focal necrosis. This change in the liver cord cells was much more constant than fatty degeneration spoken of by Poincaré in 1879, and since then by Dublin and Lieboff, Smith,²² Weese,²⁶ and others. The almost constant change in the kidney was a parenchymatous degeneration of the epithelium lining the convoluted tubules. This finding was also confirmed by Poincaré, Weese, Smith, and by Isaacs.¹⁰

In the thoracic cavity both the lungs and heart showed rather constant evidence of pathological change. In some cases the lungs gave evidence of a terminal pneumonic consolidation as noted by Weese in mice. In the milder cases edema, congestion, and desquamation of alveolar epithelium was noted, as mentioned by Pierce and by Poincaré. These changes were observed more frequently in those animals subjected to inhalation of alcohol fumes than in those treated by either absorption or by oral administration. The earliest changes in the heart began as an edema and progress to granular degeneration, and in some in-

stances, to a final necrosis of the heart muscle fibers. These findings also confirmed those of Poincaré.

The hemopoietic system and blood showed interesting changes. The earliest reference found to any change in the blood and blood vascular system is in a paper by Buchner, Fuchs, and Megele,² published in 1901, in which they discussed the capillary engorgement produced by the external application of methyl, ethyl, and propyl alcohols. Twelve years later Miura¹⁶ injected methyl alcohol into the blood stream of four rabbits and one dog. From his results he concluded that methyl alcohol is toxic to the blood-forming organs. The next year, after experimental poisoning by inhalation, Tyson and Schoenberg²⁵ stated they believed methyl alcohol to be a true hematotoxic. All the degenerative changes observed in other tissues they explained on the basis that the interference with the circulation of the blood deprived them of their nutrition and oxygen. In Smith's recent paper he mentioned splenic engorgement and phagocytosis.

In the present series the lymph nodes were frequently found to be hyperplastic and the spleen in many cases gave evidence of increased blood-forming activity. This phase of the toxic action of methyl alcohol opens a somewhat new and interesting field of investigation upon this subject and upon which we hope to make further report at an early date.

The influence of methyl alcohol upon the central nervous system was much the same as elsewhere in the body: capillary congestion, edema, and patchy degeneration in the neurones. This cellular degeneration occurred more often in the spinal cord than in the brain. Pierce recorded cerebral congestion occurring both in his own cases and in those of Strohmberg. Isaacs noted in his collection of human cases edema and congestion of the brain and meninges and an increase in the amount of spinal fluid. Rühle²¹ found in dogs, scattered hemorrhages along the blood vessels in the pons, medulla, and cord. He also noted that the vascular endothelium contained large amounts of lipoid and that there was perivascular infiltration. This condition preceded the hemorrhages. In the experiment under discussion there was no evidence of either the perivascular infiltration or hemorrhages noted

by this investigator. Holden also described hemorrhages into the meninges, brain, and cords of dogs. He agreed with several other investigators that the alcohol causes pathological changes in nerves other than the optic nerve. He reported that the experimental dogs appeared partially deaf as well as blind before death. Both Rosenau and Tyson refer to hearing impairment in human cases. Tyson also cited one case of paresis of the left internal rectus muscle of the eye. Jelliffe¹¹ has noted other forms of neuritis than optic neuritis, citing a human case with neuritis of the upper extremities. McCord, in his clinical observations on the animals which comprise this study, noted evidence of such peripheral nerve involvement. These clinical observations were later confirmed in stained preparations of peripheral nerve of these animals.

In studying the eye and optic nerve constant changes were found both in retina and nerve. However, the changes in the retina predominated and were uniformly of the nature of an acute toxic lesion. The vessels of the choroid were markedly congested. The entire retina was edematous, but especially the fiber and ganglionic cell layers. The ganglion cells were degenerated. This degeneration was patchy in occurrence, normal areas being immediately adjacent to markedly degenerated ones. These degenerated areas were not confined to any one area of the retina but were scattered throughout. These retinal changes are quite similar to those found by MacDonald¹³ in human cases, and by Smith, Weese, Holden, Friedenwald,⁵ Tyson and Schoenberg,²² and Birch-Hirschfeld¹ in experimental animals. Hale⁶ discussed Moulton's two cases of complete optic atrophy without changes in the retina. On the other hand it seems not uncommon to find degenerations in the retina without any degenerative changes in the optic nerve. Birch-Hirschfeld definitely stated that degenerations in the optic nerve occurred in but one of his cases. MacDonald did not find any optic nerve pathology in his human cases. Weese and Tyson and Schoenberg fail to mention the optic nerve. Very few of our animals showed any change in the optic nerve although numerous specific neurological stains were used. The animal showing such changes most definitely was the monkey which was blind at death.

With these findings in mind the discussion arises as to whether the retina or optic nerve is affected first, or whether they might both be the result of the same change and occur simultaneously. Tyson believed that the loss of function is due to the affected nerve fibers first or at least coincidentally with the degeneration in the ganglion cell layer. However, our observations in the present study force us to agree with Birch-Hirschfeld, MacDonald, and Henderson and Haggard⁷ in thinking that the degeneration of the retinal ganglion cell uniformly precedes any degenerative changes in the optic nerve.

The most important factor in this process is the direct action of the alcohol itself, while a contributing factor may be the accompanying edema. That such a rôle might be played by edema is suggested in a bulletin published by the National Research Council of Canada.¹⁹ Kasaas¹² believed that circulatory disturbances in the choroid and in the sheath of the optic nerve, together with edema, lead to degeneration of the retina. The author stated that the edema disappeared and visual power partially returned if collateral circulation be restored. In human cases of a chronic nature, several of recurrent transitory blindness with a gradual impairment of sight are reported in a bulletin issued by the New York State Department of Labor,³ and in some animals of the present series blindness was apparently transitory. One monkey in particular was blind both clinically and upon ophthalmoscopic examination at one time during the experiment, but prior to death its sight was proved again normal by the same means. Histological examination of the eyes of this animal revealed no evidence of pathologic change. Such transitory blindness was not of uncommon occurrence among the animals reported by McCord.

There has been a gradually growing tendency to consider methyl alcohol as a specific poison to the optic nerve and retina. This concentration of attention upon one manifestation of a disease to the exclusion of its other less conspicuous lesions is not confined to this disease, however. Among rather numerous possible examples we may cite the fact that until the very recent careful observations of Herrick⁸ the thought prevailed that the

pathology of meningococcic infection was limited entirely to the meninges. The proof that such infections are true cases of generalized septicemia has thrown new light upon this disease and explains the not infrequent overwhelming infections of this organism and sudden deaths occurring before meningeal pathology has developed. Other conditions in which a similar attitude has been assumed by the physician are the relationship of the kidney to hypertension, and also in the lesions of poliomyelitis. Similarly the lesions of the eye have been so conspicuous as to mask the other manifestations of the poison of methyl alcohol. The pathological conditions arising from these associated effects may be, however, just as constant and possibly more important than the changes taking place in the eye. Also it is possible that an individual may suffer from methyl alcohol poisoning in severe degree and still show no eye changes, just as meningeal lesions are no longer considered essential for the diagnosis of meningococcus infections.

SUMMARY

A histopathological study has been made of forty-one monkeys, thirty-six rabbits, and eighty-one rats which had previously been treated with methyl alcohol. The alcohol was administered by inhalation, by skin absorption, and orally. The method of application made no apparent difference either in the type of lesions or in their severity.

There is some variation in species susceptibility to methyl alcohol in the animals studied; rats are most susceptible to the alcohol and rabbits are most resistant. Individual variations in susceptibility to this toxin are very marked.

The characteristic pathological lesions of methyl alcohol poisoning are degenerative ones. Only the parenchymal tissues and neurones are affected. This granular degeneration of parenchymal tissue may proceed to necrosis. There is practically never injury to connective tissue and therefore seldom any fibrosis. These changes are accompanied by a generalized capillary engorgement and edema.

The pathological conditions in the eye are identical with those

found elsewhere in the body. The edema is most marked in the retina and optic disc. Degeneration of the ganglion cells of the retina is sometimes followed by degenerative changes and fibrosis within the optic nerve.

There may be multiple degenerations involving nerves other than the optic nerve.

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EDITORIAL

THE BENDIEN TEST

Bendien's book, *Spezifische Veränderungen des Blutserums*, published in 1931, was translated the same year by A. Piney. There may be academic interest in Bendien's method of approach to various problems. However, his chief claim for recognition is on the grounds that his test has value in the diagnosis of cancer. Bendien's reagents are made up with varying quantities of tenth normal acetic acid, and of tenth normal sodium vanadate. These various mixtures provide twenty different solutions with very slight differences in hydrogen ion concentrations from pH 3.3 to 5.4. Five cubic centimeters of each reagent is used in the test, which requires twenty tubes. In each of these tubes, before adding the reagent, there is placed 0.5 cc. of clear serum and 0.5 cc. of distilled water. After standing twenty-four hours at room temperature flocculation is found to occur in some of the twenty tubes. In certain diseases, notably carcinoma, active tuberculosis, or obstructive jaundice, precipitation may occur in the first or second tube, pH 3.3. As the solutions become slightly less acid, precipitations occur with serums from patients with other diseases, diabetes, pleurisy, pregnancy, malaria, nephritis, furunculosis, scarlet fever, syphilis, and so forth. Bendien was not satisfied with such a simple procedure as that briefly outlined but increased the technical difficulties by filtering out some of the precipitates, weighing the residue and making a 1 per cent NaHCO_3 . This was then studied spectrophotometrically.

Meyer reported unfavorably on the test and stated that it is not specific in any way. Smith, Holiday and Marrack investigated the spectrophotometric details of the test and concluded that the method is valueless in the diagnosis of cancer. Such was also the conclusion of Freeman. A recent advocate for the test, Lowe, has complicated the technic further by studying reactions of heated and unheated serum and by dissolving the precipitate in hundredth normal sodium hydroxide and then using a Löwe interferometer. It is not necessary to discuss the some-

what involved estimations that must then be made by means of which a "reaction formula" is evolved, placing the serum either in a malignant or nonmalignant category. Lowe referred to criticisms of Bendien's original method and claimed superiority for his own method. It does not seem, however, that Lowe has entirely answered the criticism of Fine, who stated: "The Bendien chemical test has no real value in diagnosis. It has certain value in prognosis, probably not greater than that of the clinical signs and symptoms present. In its mechanism and in the information it supplies it is identical with the sedimentation rate test, to which it has not been proved superior." Fine's explanation for the early reports of 100 per cent accuracy in the diagnosis of cancer is: "when a batch of bloods is supplied containing a certain number derived from inoperable carcinomas, the remainder being from mild, non-wasting, and non-suppurative conditions, those from carcinoma alone give a precipitate in tubes 1 and 2. Such success, though of no practical application, produces an ephemeral enthusiasm for the test—a phase I passed through myself."

It is of course greatly to be desired that some sharply differential test be devised for the early diagnosis of cancer. It is barely possible that Lowe's modification of Bendien's test may have made this method more useful, but the weight of the evidence is still on the side of those who hold the opinion that this method cannot yield specific results.

A. H. SANFORD.

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NEWS AND NOTICES

THE TWELFTH ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

The Twelfth Annual Convention of the American Society of Clinical Pathologists was held in Milwaukee, Wisconsin from June 9 to 12. The meetings were well attended and as was anticipated the Symposium on the Medico-Legal Autopsy was a tremendous success. Due to popular demand it has been decided to issue the Symposium as a monograph after the papers have been published in the JOURNAL. The rest of the Scientific sessions contained papers of great merit and many valuable contributions were made.

The details of the business meeting will be published in a subsequent issue of the JOURNAL.

The following officers were elected and committees appointed:

President-Elect: Dr. F. H. Lamb

Vice-President: Dr. J. J. Seelman

Secretary-Treasurer: Dr. A. S. Giordano

Members of the Executive Committee:

Dr. W. M. Simpson (3 years)

Dr. H. C. Sweany (3 years)

Dr. J. H. Black (1 year)

Members of the Board of Censors:

Dr. J. J. Moore (3 years)

Dr. A. V. St. George (2 years)

Dr. C. W. Maynard (3 years)

Members of the Board of Registry:

Dr. R. R. Kracke (3 years)

Dr. H. H. Foskett (3 years)

The following were elected to membership:

Ball, Howard A.
Balle, Alfred L.
Barret, Harvey P.
Bates, Lewis Beals
Bleyer, Leo Frederick
Braunstein, Wm. P.
Bugher, John C.
Case, Lucius W.
Culbertson, Clyde G.
Dobos, Emeric I.
Edgar, Jas. D.
Elton, Norman W.
Evans, Newton
Goforth, John L.
Gordon, Harold
Heck, F. J.
Hellwig, Christian
Howard, Stacy C.
Kemp, Hardy A.
Kitzmiller, Karl Vivian
Konzelmann, Frank W.
Kosky, Alfred A.

Leichliter, John W.
Lewis, Seaborn J.
Norris, Jack C.
Oesterlin, Ernest J.
Parker, Marian E.
Pessin, Samuel B.
Pons, Carlos A.
Reinhart, Harry L.
Roth, Joseph F.
Secoof, David Philip
Shafer, Rudolph J.
Shirey, Ralph W.
Stout, Sidney E.
Summerlin, Harold S.
Thorsness, Edwin T.
Tripoli, Carlo J.
Wallace, Stuart A.
Weeter, Harry M.
Wenner, Thos. J.
Williford, Herman B.
Wright, Arthur Wm.
Young, Anna May

T. B. MAGATH.

BOOK REVIEWS

Practical Hematological Diagnosis. By O. H. PERRY PEPPER AND DAVID L. FARLEY. Pp. 562, 1933. London, Philadelphia, W. B. Saunders Company, \$6.00.

This book was primarily designed to lessen the confusion which has arisen due to the numerous terms and methods employed in clinical hematology. The authors desired to present the practical aspects of hematology in simple terms. To this end they have succeeded very well although it is evident that the subject is entirely too complicated to ever hope to make a very simple discussion.

The book is divided into three parts, the first of which covers the components of the blood, the methods for their study and the practical significance of the results obtained. After discussing the cellular elements with little or no reference to the serum and plasma, chapters are devoted to coagulation of blood, blood groups and parasites of the blood.

The second part deals with hematologic findings in the diseases and disorders of the hematopoietic system with chapters on the anemias, polycythemia, the leukemias, purpuric and hemorrhagic diseases, the hematology of infancy and the effects of irradiation and splenectomy.

The last part is concerned with the hematologic findings of diseases not primarily of the blood and consists of an alphabetical list of essential blood pictures in a large list of diseases. The closing chapter is a glossary of hematologic terminology. Throughout the text numerous references appear covering the major recent contributions to the subject.

The general and specific discussions are brief and clear and will appeal primarily to the clinician. Methods were selected arbitrarily although in some instances as for example hemoglobin, numerous methods are given. In this respect the authors have not made the book so practical. Although a half page is devoted to the use of buffers in staining, the formula for the buffer is not given. No method is given for reticulocyte staining and Wright's staining technic is the only one to which reference is made. No

mention of the preparation of blood smears on slides can be found, the coverglass method, which is practical only in the hands of experts, is the only one indicated. All the recent elaborate work on the errors of differential counting is omitted. The prothrombin time test, given in detail, is without reference to Howell's own criticism of his original paper and blood volume is too briefly mentioned for the reader to appreciate any of the problems involved.

While these may or may not be valid criticisms of the text, the book will be useful to clinicians who cannot possibly delve into the intricacies of the subject in the way that clinical pathologists and specialists in the field have found it necessary.

Lymphatics, Lymph and Tissue Fluid. By CECIL K. DRINKER AND MADELEINE E. FIELD. Pp. vii + 254, 1933. Baltimore, The Williams & Wilkins Company. \$3.00.

Important as the lymphatic system is there have been very few comprehensive monographs written on the subject. Physiologists, anatomists, pathologists and internists will therefore be delighted to have at hand a modern treatise, brief yet comprehensive, dealing with this difficult subject. The monograph has come as the result of seven years intensive study of the lymphatics and lymph and deals with the structural basis of the lymphatic system, the entrance of foreign particles and colloidal solutions into lymphatics, the permeability of the blood capillaries, the limits of the permeability of the typical normal blood capillary to colloids, the flow of lymph, the composition of lymph, the tissue fluid and finally practical considerations involving a host of clinical questions. The style of the book is excellent and extremely clear, the reader at no time being in doubt as to the status of a particular problem at the present time. This feature is made very clear by an unusual author and bibliography index. From this one can tell at once where to find any reference to previous work in the text. The authors have interpolated numerous tables which briefly and completely describe the various components of the lymph and the results of numerous experiments such as the effect of drugs, salts, extracts, position of the animal, blood pressure, etc.

This is surely an important monograph and must be available to serious students of medical subjects.

THE CLINICAL PATHOLOGIST AS CONSULTANT AND TEACHER*

WALTER M. SIMPSON

Director, Diagnostic Laboratories, Miami Valley Hospital, Dayton, Ohio

"The practice of clinical pathology offers to the physician an unusual opportunity. It keeps him in intimate touch with disease in all its phases. It is a stimulus to medical research. It is scientific and accurate beyond the science of history taking or physical diagnosis. It is one of the most valuable aids to modern medical practice. American physicians are fortunate to have the assurance that the practice of clinical pathology is being maintained on a high plane." This quotation from an editorial which appeared in a recent number of the *Journal of the American Medical Association* carries with it a ringing challenge to the members of the American Society of Clinical Pathologists to strive vigorously to make the field of clinical pathology an even greater asset to the practice of modern medicine. To those who nurtured this Society in its infancy these words of praise should provide recompense for their relentless efforts to elevate the practice of clinical pathology to its present high plane.

A decade ago, when the idealism of Ward Burdick created this Society, the field of clinical pathology was an ill-defined territory in medical practice. In fact, during the first few years of the Society's growth some difficulty was encountered in arriving at a satisfactory definition of a clinical pathologist. Today, the clinical pathologist is recognized as a consulting physician whose chief interest lies in the diagnosis of disease by laboratory methods.

There may be a few persons who still think of the clinical pathologist as a glorified, overpaid technician. If there remains

* Presidential address read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9-12, 1933.

a clinical pathologist who still feels that he possesses such a status, he has only himself to blame. The day has long since passed, if in fact it ever existed, for the clinical pathologist to find it necessary to endear himself to one or two surgeons in order to hold his job. The growth of clinical pathology is in some respects not unlike that of surgery. Not so many decades ago the surgeon enjoyed the social status of a barber. Thanks to a dentist and a chemist, an opportunity was provided for a dominating intellectual group to bring the technic of surgery to its present perfection. It must be apparent to all that the heyday of surgery as the dominant field of medical practice has passed and that internal medicine and diagnosis are again destined to occupy the leading rôle. The recent strides in surgery have been largely due to the recognition by the surgeon that he can no longer travel alone, but that he must seek the collaboration of the physiologist, the pathologist, the bacteriologist, the biochemist and the physicist.

The renewed emphasis on diagnosis and rationally controlled therapy has brought the clinical pathologist to a place of prominence in medical practice. The past few years have witnessed an interesting blending of the fields of internal medicine and clinical pathology. The modern internist must possess an intimate working knowledge of the diagnostic and therapeutic aids provided by the clinical pathologist. By that same token, the clinical pathologist has been brought from the seclusion of his laboratory to the patient's bedside. The opinion of the modern clinical pathologist now ranks with that of any other medical consultant. It is natural, therefore, that as the field of clinical pathology continues to widen, the broad knowledge of the adequately trained clinical pathologist in the fields of hematology, serology, bacteriology, biochemistry, and histopathology will be more and more in demand by private practitioners in all fields of medicine. This natural evolution makes it imperative that the clinical pathologist should be thoroughly versed in clinical medicine. Without this background the information gained by laboratory studies is subject to misinterpretation and consequently does not redound to the welfare of the patient. That the clinical pathol-

ogist, whose long period of special training often exceeds that of many physicians in other special fields, should be compensated to the same extent as any other consultant, is a matter of simple justice.

With this growth of opportunity comes a greater degree of responsibility. The constant addition of new laboratory procedures demands the delegation of much of the purely technical work to lay technicians. Thanks to the unselfish and untiring labors of Dr. Philip Hillkowitz and the other members of the Board of Registry of Technicians of our Society, together with the cordial support of the Council on Medical Education of the American Medical Association and the American College of Surgeons, safeguards have been erected to prevent further invasion of the field by incompetent lay workers. It is the duty of all clinical pathologists who seek the respect and confidence of their medical brethren further to strengthen their position by eliminating inadequately trained or disinterested or dishonest lay technicians, and insist upon the certification of all capable workers by the Board of Registry.

The ever-widening scope of the field of diagnosis by laboratory investigation carries with it some danger of neglect of what Dr. Kenneth Lynch has aptly termed "the keystone of the practice of clinical pathology," namely, pathologic anatomy. Deliberated judgment, resulting from twenty years experience as a teacher of general pathology, director of a teaching hospital division of pathology and in the private practice of clinical pathology led Dr. Lynch to state that six years of training in tissue diagnosis is productive of less competency than six months in the other branches of clinical laboratory practice. With this thesis I am in complete accord. A thorough knowledge of gross and microscopic pathologic anatomy is perhaps the greatest single distinguishing characteristic which commands the respect of a clinical pathologist by his medical fellows. If we are to adhere for the present to the stipulation that a clinical pathologist is a physician who has "specialized in clinical pathology, bacteriology, pathology, chemistry, or other allied subjects for at least three years subsequent to graduation, who is in good standing and has been duly

licensed to practice medicine" (as defined by the Council on Medical Education and Hospitals of the American Medical Association) it seems reasonable to expect that at least two of the three years should be spent exclusively in pathologic anatomy. The objections which have been raised by various organizations, particularly in the diagnosis of neoplastic disease, would then be unjustified. The clinical pathologist must always remain a student. A month or two of special study of neoplasms every two or three years would be a profitable investment. Some of our own Fellows in the universities and larger clinics might consider the advisability of instituting such seminar courses, not only in the field of neoplasia, but also in hematology, serology, bacteriology and biochemistry.

Since it is now firmly established that the modern clinical pathologist is preëminently a consultant to other members of the medical profession there appears to be little excuse for the maintenance of such consultants at public expense. It would be as sensible to maintain at the expense of the taxpayer consulting surgeons, consulting pediatricians or consulting internists at the state capitol. Possibly the greatest excursion into the dark uncharted sea of state medicine has come as a result of the extraordinary development of certain state health laboratories, not infrequently under the direction of a person whose qualifications do not conform to the accepted definition of a clinical pathologist. The influence of the professional social worker has been an important factor in this unfortunate development. Since the publication of the report of the Committee on the Costs of Medical Care it has become apparent to practically every physician and to many sociologists that the time has come for the social workers to give the practice of medicine back to the doctors. The sensible and logical curtailment of the activities of the state health laboratories of Indiana provides a program for action by the Fellows of this Society in other states. When clinical pathologists in hospital and private practice have convinced the state medical associations that a higher type of service can be rendered by the physician-pathologist, the encroachment by state health laboratories on the field of clinical pathology will cease and the

state laboratories will revert to the purpose for which they were originally designed, namely, the control of transmissible disease and the provision of a diagnostic service for the indigent sick.

The clinical laboratory has become the scientific keystone of the modern hospital structure. It has been stated that "a hospital is only as big as its department of pathology." The hospital laboratory is the axis about which the scientific work of the hospital revolves. Upon the clinical pathologist, therefore, devolves the responsibility for the correlation and the dissemination of the scientific aspects of hospital medical practice. For this reason the clinical pathologist must be a teacher. The teaching activities of the alert clinical pathologist may assume several forms. The cheerful willingness correctly to interpret laboratory findings is one of the most fruitful forms of medical teaching, particularly as related to the welfare of the patient. The hospital staff conference and the clinico-pathologic conference provide perhaps the best method for the pathologist to contribute a large share to postgraduate medical education. The well-directed clinico-pathologic conference makes available the university idea of postgraduate medical education in every hospital with a capable clinical pathologist. He who does not fulfill this duty is missing his greatest opportunity to gain the respect and confidence of his medical brothers. Such conferences are capable of providing the greatest single stimulant to the scientific activities of the hospital. The intelligent correlation and interpretation of the information gained by postmortem examinations will quite naturally create interest in morbid anatomy and the clinician is provided with a real incentive to obtain permission for post-mortem examinations on his patients.

The successful conduct of clinico-pathologic conferences requires consummate tact. If the emphasis is placed too heavily upon postmortem pathology, at the expense of clinical aspects, the interest of the average practitioner will not be aroused. The practice of some hospitals to make attendance at the clinico-pathologic conference compulsory to all staff members should be condemned. Attendance at the clinico-pathologic conference should be optional and induced only by the excellence of the

program. Such conferences should be held often enough to sustain interest. The weekly or bi-weekly clinico-pathologic conference, lasting for an hour or an hour and a half, but not longer, will accomplish this end. The logical place for the conference is in the hospital. The time for the conference should be chosen with a view to inconveniencing the smallest number of persons. The programs should be arranged so that a wide variety of subjects might be discussed at each conference, in order that the physicians in special fields might be induced regularly to attend the conferences. Recurrent discussion of the simple, commonly encountered diseases is of much greater importance than the presentation of rare and unusual diseases. The clinical pathologist is the natural and logical director of such conferences.

While it is generally admitted that the pathologist is usually "the court of last appeal," the shrewd and tactful pathologist will rarely exercise this prerogative. To give instruction to the informed physician is quite a different matter than the teaching of the uninformed layman or medical student.

Perhaps the greatest single asset to the clinical pathologist is an unfailing sense of humor. The shrewd clinical pathologist is constantly aware of the fact that physicians represent the greatest group of individualists of any professional field of endeavor. The exercise of restraint will accomplish much more than a practice of forcing his opinions and decisions upon his fellow practitioners.

The position of the clinical pathologist as consultant and teacher now rests upon a firm and substantial foundation. The evolution of medicine has placed the clinical pathologist in a commanding position. Much as the Council on Medical Education of the American Medical Association has raised the level of medical education to its present high plane, and as the American College of Surgeons and the American College of Physicians have succeeded in elevating the standards of surgical and medical practice, so the American Society of Clinical Pathologists is performing in a forceful manner much the same function as regards the elevation of the clinical pathologist to his rightful heritage.

THE AGGLUTININ CONTENT OF THE BLOOD FOLLOWING TYPHOID AND PARATYPHOID IMMUNIZATION*

ALVIN G. FOORD AND ANNA FORSYTH

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Most clinical pathologists have seen patients sent to their hospitals with a diagnosis of typhoid fever because of the presence of an otherwise unexplained fever and a positive Widal test. On further study some of these patients have been found to be suffering from another disease and the Widal reaction has been due to a previous vaccination. The widespread use of vaccination in military, school, and hospital circles, and even in the general population, has multiplied this rather serious source of error in recent years so that controlled observation of the agglutinin reactions of the blood of immunized persons are desirable. Recent literature reveals little information as to the duration or the strength of agglutinins in the blood following vaccination. However, Karl Meyer and Kilgore³ report persistence of agglutinins for a period of ten months and summarize the literature up to the year 1917. They properly criticize much of the older work because of the technique employed, and the qualitative and quantitative variations of the antigens used both for vaccination and for the agglutination tests. More recently Hoffstad¹ and Thompson² report that in a series of persons immunized by the oral route, agglutinins persisted in a fair proportion of cases for five and fewer for nine months. Tests at later periods following vaccination were not reported by the above authors. Chambers¹ however, tested bloods from a series of war veterans vaccinated at various camps during the war and found appreciable agglutination in about half the cases for as long as thirteen years after inoculation.

* Read before the Eleventh Annual Convention of the American Society of Clinical Pathologists, New Orleans, Louisiana, May 6-9, 1932.

In the experiments herein recorded the agglutinin content of the blood of 120 vaccinated nurses and internes of the Buffalo City Hospital was determined in a period of two days, using the same flask of antigen for all the tests. Inasmuch as all the nurses were vaccinated by one of us (A.G.F.) with triple vaccine of the same type on their entrance into training, we were able to obtain bloods from small groups from the various classes immunized at periods varying from twenty-three days to nine years after the date of the last of three weekly injections.

METHOD OF OBSERVATION

The vaccine used was the triple vaccine supplied by the New York State Board of Health Laboratory, who obtained the strains from the Army Medical School, and contained one billion typhoid (Rawlins) and 750 millions each of paratyphoid A (Kessel) and paratyphoid B (Rowland) bacilli per cubic centimeter. The doses were 0.5 cc., 1 cc. and 1 cc. given subcutaneously at weekly intervals. All received three full doses. None had had typhoid fever or previous inoculations. The antigen used in the agglutination tests was prepared in a two liter flask of Liebig extract broth containing 0.3 per cent meat extract, 0.5 per cent sodium chloride and 1 per cent Witte's peptone, adjusted to give a pH of 7.3 after autoclaving, to which was added sufficient typhoid bacilli (N. Y. State No. 305) to yield a satisfactory growth. After eighteen hours incubation the culture was killed by adding 1 cc. of 40 per cent formaldehyde per liter of antigen and allowed to stand in the icebox for two weeks before using. Control tests showed proper agglutination with standard serum supplied by the New York State Laboratory and no agglutination at dilutions of 1:20 or above, with serums of normal non-inoculated individuals.

The serums to be tested were inactivated in a water bath at 56°C. for half an hour, and dilutions of 1:10, 1:20, and upward by multiples of two to include the anticipated agglutination range, and to 0.5 cc. of each dilution 0.5 cc. of the antigen was added. The tubes were placed in a water bath at 38°C. for two hours, and read after standing in the icebox overnight. The titers given in table 1 represent the highest dilution at which agglutination was visible macroscopically.

Table 1 shows the number of serums agglutinating at the various highest dilutions at the different time intervals following the last of three injections. All of the serums studied show agglutinins ranging from a titer of 1:20 to 1:1280. The titer showed an average of 1:320 to 1:640 at the end of twenty-three days and 1:80 to 1:160 at the end of eleven months, but agglutinins were

TABLE 1

NUMBER OF SERUMS AGGLUTINATING TYPHOID BACILLI AT VARIOUS DILUTIONS
AT DIFFERENT TIME INTERVALS AFTER VACCINATION

| TIME | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 | 1:1280 | TOTAL SERUMS |
|-----------------|------|------|------|-------|-------|-------|--------|-----------------|
| 23 days | | | | 3 | 10 | 8 | 1 | 22 |
| 32 days | | | | 4 | 5 | 3 | | 12 |
| 4½-5 months | | | 4 | 8 | 2 | 4 | 2 | 20 |
| 9 months | | 2 | 4 | 6 | 3 | 3 | | 18 |
| 10½-11 months | | 1 | 2 | 2 | | | 1 | 6 |
| 18 months | | 2 | 2 | 6 | 1 | | 1 | 12 |
| 2 years | | | 1 | 1 | | | | 2 |
| 26 months | 1 | | 3 | 2 | | | | 6 |
| 2½ years | | | 3 | 3 | 1 | | | 7 |
| 3 years | | | 2 | | 1 | | | 3 |
| 3½ years | | | 2 | 1 | | | | 3 |
| 4 years | | 1 | 1 | 1 | | | | 3 |
| 5 years | | | 2 | | 2 | | | 4 |
| 6 years | | | | 1 | | | | 1 |
| 9 years | | | | 1 | | | | 1 |
| Total serums... | | | | | | | | 120 |

TABLE 2

AGGLUTININ TITERS OF TWENTY-THREE SERUMS AGAINST TYPHOID AND PARA-
TYPHOID BACILLI AT VARIOUS TIME INTERVALS

| SERUM | TIME | TYPHOID | PARATYPHOID A. | PARATYPHOID B. | SERUM | TIME | TYPHOID | PARATYPHOID A. | PARATYPHOID B. |
|-------|-----------|---------|----------------|----------------|-------|-----------|---------|----------------|----------------|
| 1 | 4½ months | 1:1280 | 1:20 | 1:160 | 13 | 9 months | 1:160 | 1:20 | 1:80 |
| 2 | 4½ months | 1:640 | 1:40 | 1:40 | 14 | 11 months | 1:1280 | 1:80 | 1:80 |
| 3 | 4½ months | 1:640 | | 1:80 | 15 | 18 months | 1:160 | 1:20 | |
| 4 | 4½ months | 1:160 | 1:20 | 1:40 | 16 | 18 months | 1:160 | 1:40 | 1:40 |
| 5 | 4½ months | 1:160 | 1:20 | 1:40 | 17 | 18 months | 1:40 | | 1:20 |
| 6 | 4½ months | 1:160 | 1:20 | 1:40 | 18 | 30 months | 1:160 | 1:40 | 1:80 |
| 7 | 4½ months | 1:160 | 1:20 | 1:80 | 19 | 30 months | 1:80 | 1:40 | 1:40 |
| 8 | 4½ months | 1:160 | 1:40 | 1:320 | 20 | 3 years | 1:80 | 1:40 | 1:160 |
| 9 | 4½ months | 1:80 | 1:40 | 1:40 | 21 | 3 years | 1:80 | | 1:160 |
| 10 | 9 months | 1:320 | 1:40 | 1:160 | 22 | 4 years | 1:80 | 1:40 | 1:40 |
| 11 | 9 months | 1:160 | 1:80 | 1:80 | 23 | 5 years | 1:80 | 1:20 | 1:40 |
| 12 | 9 months | 1:160 | 1:20 | 1:80 | | | | | |

present in clinically important dilutions for a period of many years, including one serum agglutinating at 1:160 at nine years and one at a similar dilution six years after immunization.

A series of twenty-three sera were tested for agglutinins for paratyphoid A (N.Y. State No. 17) and paratyphoid B (N. Y. State No. 18) bacilli with antigen prepared in a method similar to that outlined for the typhoid strain mentioned above. The results in table 2 show that on the average there was less agglutinin formed against the paratyphoids than against typhoid bacilli, and usually higher paratyphoid B titers were produced than paratyphoid A. However, there was no definite regularity as to the response to the three organisms since some serums showed high typhoid titers and low paratyphoid titers, and in an occasional case this relation was reversed.

SUMMARY AND CONCLUSIONS

Agglutination tests conducted simultaneously with the same flask of antigen on serums of 120 nurses and internes immunized by three weekly subcutaneous injections of triple typhoid paratyphoid A and B vaccine furnished by the New York State Department of Health revealed the presence of typhoid agglutinins in a dilution averaging 1:320 to 1:640 at the end of twenty-three days following the last injection.

Tests at longer intervals of several months and to five to nine years demonstrated persistence of agglutinins in all the serums studied in lower, but clinically significant amount, for example, 1:160 after six and nine years, respectively.

Agglutinins against paratyphoid bacilli were demonstrated at titers averaging somewhat lower than the corresponding titer against the typhoid bacillus.

In evaluating a positive agglutination test against typhoid or paratyphoid organisms, the history of prophylactic immunization must be considered, even though many years have elapsed since the inoculations.

We wish to thank Dr. Benjamin Smallman, Miss Sophia Zurette, and Mrs. Marjorie Bauckus Cargill for their aid in preparing sera and setting up the tests.

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CHOLECYSTECTOMY AS SEEN BY THE SURGICAL PATHOLOGIST

REPORT OF 223 CASES

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The types of pathology which, occurring in the gallbladder, have given rise to characteristic symptoms and to surgical removal of the gallbladder, have been described in detail by Baumgartner.¹ Becker² and Mentzer⁵ have each reported the incidence of gallbladder lesions in a series of postmortem examinations. Surgeons have presented their view of the indications for cholecystectomy repeatedly, and some have told of the results obtained subsequent to this operation. I have not found reference to a surgical pathologist presenting the question of why normal gallbladders appear in his laboratory, or what their significance may be.

In studying this series of 223 gallbladders which have come to me from nineteen surgeons in five hospitals, I have tried to correlate preoperative diagnoses, pathological findings, and clinical results. The removal of appendices, and the operative treatment of peptic ulcer, as these have been done in addition to the removal of the gallbladder have also been noted. The surgeon who is irritated when the pathologist fails to find sufficient evidence of disease in the removed gallbladder, the pathologist who wonders whether the surgeon is not sometimes overenthusiastic about cholecystectomy and the patient who has to live on without a gallbladder, normal or diseased, will be concerned with this study.

In histology and gross anatomy the gallbladder differs somewhat from the appendix, with which it competes as a cause of digestive disturbance which can be removed by surgery. Its mucosa contains no lymphoid tissue, and such mucus as is fur-

nished to lubricate its surface comes from the columnar cells which cover it, rather than from definite gland structures. The greater part of the wall consists of a fibromuscular layer, in which poorly developed bundles of smooth muscle interlace in a rather loose fibrous stroma. The serous and subserous layers differ in no way from the same tissues throughout the peritoneal cavity.

The gallbladder is not subject to the gross fecal contamination which carries anaerobes and a multitude of more or less virulent bacteria to the appendix. As a result acute inflammations are comparatively rare in the right upper quadrant of the abdomen. On the other hand, the gallbladder is, as a storage reservoir, subject to mechanical obstruction of its exit, and to precipitation of salts from its contents. These more or less continuous irritants are at least partially responsible for the presence of scattered lymphocytes in the gallbladder wall, and for the slowly increasing predominance of fibrous tissue over muscle, which we know as chronic cholecystitis. Bacteria, entering from liver, duodenum, lymph or blood stream, may combine with the mechanical effect of stones, and produce acute lesions. Malignancy develops occasionally.

Making the data as simple as possible without sacrificing the accurate accomplishment of our purpose, I have grouped the cases in this series, according to the preoperative diagnoses, as (1) chronic cholecystitis (without stones); (2) cholelithiasis; (3) acute cholecystitis, and (4) peptic ulcer. The demonstrable pathology is divided into (1) chronic cholecystitis; (2) cholelithiasis; (3) carcinoma, and (4) no pathology found in the gallbladder. The fourteen cases of acute inflammation, and nine of papilloma, have been placed with the chronic conditions upon which they have been superimposed.

The records show that diagnoses have been made from the usual symptoms, physical findings, and X-Ray reports, with or without the use of dye tests. Gallbladders have been removed after a diagnosis of peptic ulcer chiefly for three reasons, gross thickening of the wall, the presence of adhesions about the gallbladder, and failure of the viscus to empty easily under compression.

The comparison of clinical and pathological findings is shown

in table 1. One hundred sixty-three cases were diagnosed "chronic cholecystitis," or "gallbladder disease." Of these, ninety-seven showed infiltration with lymphocytes and/or the

TABLE 1

| PREOPERATIVE DIAGNOSIS | PATHOLOGICAL DIAGNOSIS | | APPENDECTOMIES | | | |
|--|---|-----|----------------|-------|---------|----------------------------|
| | | | Normal | Acute | Chronic | Previous opera- tion |
| Chronic chole- cystitis (gall- bladder dis- ease), 163 cases | Chronic cholecystitis..... | 97 | 10 | 1 | 33 | 18 |
| | Peptic ulcer..... 1 | | | | | |
| | Papilloma..... 7 | | | | | |
| | Acute inflammation.. 4 | | | | | |
| | Cholelithiasis..... | 46 | 6 | 0 | 10 | 2 |
| | Acute inflammation. 6 | | | | | |
| | Carcinoma..... 1 | | | | | |
| | Carcinoma..... | 2 | 0 | 0 | 1 | 0 |
| | Epidermoid..... 1 | | | | | |
| | Adenocarcinoma.... 1 | | | | | |
| Cholelithiasis, 36 cases | No pathology found in gallbladder..... | 18 | 5 | 2 | 7 | 2 |
| | Chronic cholecystitis..... | 3 | 1 | 1 | 0 | 0 |
| | Cholelithiasis..... | 32 | 4 | 0 | 8 | 4 |
| | Acute inflammation. 4 | | | | | |
| | Papilloma..... 2 | | | | | |
| Acute cholecys- titis | No pathology found in gallbladder..... | 1 | 1 | 0 | 0 | 0 |
| | Acute cholecystitis with stones..... | 4 | 1 | 1 | 0 | 0 |
| Peptic ulcer, 20 cases (7 found) | Chronic cholecystitis..... | 14 | 1 | 0 | 4 | 3 |
| | Papilloma..... 1 | | | | | |
| | Cholelithiasis..... | 1 | 1 | 0 | 0 | 0 |
| | No pathology found in gallbladder..... | 5 | 0 | 0 | 2 | 2 |
| Totals..... | | 223 | 30 | 5 | 65 | 31 |

fibrosis which I have called chronic cholecystitis. In addition, four of these patients had acute inflammatory changes, seven had developed papillomata in the mucosa, and one patient had a peptic ulcer in addition to cholecystitis.

In forty-six of this group gallstones were present, but had not been mentioned in the preoperative record. Six acute inflammations and one with squamous-cell carcinoma were associated with stones. In addition to the carcinoma complicating gallstones, one adenocarcinoma in a gallbladder free from stones was found. In eighteen gallbladders from this group of patients I did not find any tissue change which was considered pathological.

TABLE 2

| CLINICAL RESULTS | | PATHOLOGY | | | | | | |
|---------------------------|-----|---------------|--------|-----------|-----------|--------------|------------|------------------|
| | | Cholecystitis | Stones | Papilloma | Carcinoma | Peptic ulcer | None found | Appendix removed |
| After 1 to 10 years: | | | | | | | | |
| Cured..... | 76 | 33 | 34 | 5 | 0 | 2 | 9 | 45 |
| Improved..... | 31 | 17 | 8 | 0 | 1 | 2 | 5 | 20 |
| No improvement..... | 41 | 27 | 7 | 0 | 1 | 3 | 6 | 23 |
| Less than 1 year: | | | | | | | | |
| Improved..... | 33 | 16 | 16 | 1 | 0 | 1 | 1 | 19 |
| No improvement..... | 4 | 3 | 0 | 0 | 0 | 0 | 1 | 1 |
| Deaths within 30 days.... | 9 | 5 | 4 | 0 | 0 | 0 | 0 | 3 |
| Due to: | | | | | | | | |
| Diabetes..... | 1 | | | | | | | |
| Pneumonia..... | 1 | | | | | | | |
| Ileus..... | 1 | | | | | | | |
| Hepatitis..... | 1 | | | | | | | |
| Peritonitis..... | 1 | | | | | | | |
| Cause not given... 4 | | | | | | | | |
| Totals..... | 194 | 101 | 69 | 6 | 2 | 8 | 22 | 111 |

Among thirty-six cases diagnosed as "cholelithiasis" the agreement was much better. In only four were the stones not present, and three of these showed the changes of chronic inflammation.

The four patients diagnosed as having "acute cholecystitis" all had gallstones, and an acute inflammatory process in addition.

I have mentioned above the reasons given in the operative records for removing twenty gallbladders after diagnoses of peptic ulcer. Opinions necessarily will differ as to whether or not find-

ing five "normal" gallbladders in this group, nearly three times the proportion found in the other cases, has any significance. Graham and his associates⁴ state that pressing upon the gallbladder as a test of patency of the ducts is inadvisable; and Blond³ believes that valvelike folds of mucosa in the cystic duct may oppose violent attempts to expel the bile. Perhaps this surgical habit should be revised.

One hundred thirty-one patients, or 59 per cent of the total, lost their appendices either at the time of the cholecystectomy or in previous operations. This fact is of some interest in connection with the clinical results which followed these operations.

We have been able to follow up 194 of these patients, and secure from the histories or from the surgeons, reports as to relief from the symptoms which brought the patients to operation. Considering that a year should be allowed for the subsidence of the hepatitis which accompanies most cases of gallbladder infection, and for clinical readjustment, I have made two groups, one consisting of those patients whose condition is known for from one to ten years after operation, the other of patients operated upon since July 1st, 1931.

Fifty-one per cent report cures after one year, and 21 per cent are improved. The remaining 28 per cent, of the older group still have their original symptoms. The thirty-seven patients operated upon during the past year have been more fortunate, 90 per cent of them reporting relief from symptoms. Figuring only cases having gallstones, 84 per cent of the entire series are cured or improved. Nine of the patients have died within thirty days after operation, 4 per cent of the total series.

If we combine the records of all the patients whose present condition we know, we find that seventy-six, or 39.2 per cent are cured; sixty-four, or 33 per cent are improved; and forty-five, or 23.2 per cent are not improved.

But what of the normal gallbladders? I have found them in 12 per cent of the "cured" list, in 10 per cent of the "improved," and in 15 per cent of the unimproved: not an impressive difference. Perhaps this may be interpreted as evidence for the existence of "cholecystopathy," a condition of abnormal metabolism

without anatomical change. Certainly no facts have been brought out to discourage the surgeon from undertaking cholecystectomy when his diagnostic measures indicate probable gallbladder disease. The pathologist must agree that patients may receive symptomatic relief after the removal of gallbladders free from apparent lesions.

One other question occurs. Is it possible that coincident appendectomy or pyloroplasty helps in this cure of patients whose gallbladders have not shown disease? There have been 111 appendectomies, or 57 per cent of the number whose clinical condition was known. This compares well with the 60 per cent of the cured, 60 per cent of the improved, and equally well with the 54 per cent of unimproved, who have had the appendix removed. Such figures suggest that appendectomy may add slightly to the chance for cure, but are not convincing. Among the normal gallbladders there are fifteen which, aided by eleven appendectomies and two ulcer operations, are reported as cured or improved. Seven similar patients, from whom five appendices and one ulcer were removed, are not improved. This appears to show that the extra surgery which went with the removal of the anatomically normal gallbladders did not increase the patients' chances for recovery, nor did it lessen such chances.

One may conclude from this study that patients with gallstones have been most certain (84 per cent) to improve after cholecystectomy; and that 65 per cent of the chronic cholecystitis cases have been relieved. The general average of cures and improvement in the series is 72.2 per cent. Seventy per cent of the normal gallbladder removals were clinically successful, and this improvement was not increased by coincidental surgery of appendix or ulcer.

SUMMARY

(1) Cholecystectomies done on 223 patients are compared with respect to clinical and pathological findings, with especial attention to the result when the gallbladder has shown no anatomical pathology.

(2) Twenty-four, or 11 per cent of the gallbladders removed

failed to show any anatomical pathology, gross or microscopical. Of these, eighteen were from patients diagnosed as having chronic cholecystitis, or "gallbladder disease," one was removed under a "gallstone" diagnosis and five were from cases in which peptic ulcer was suspected.

(3) Reports were secured concerning the clinical condition of 194 patients one year or more after operation, or up to July 1st, 1932. Of these, seventy-six, or 39.2 per cent are reported cured, sixty-four, or 33 per cent improved and forty-five, or 23.2 per cent not improved. The mortality within thirty days after operation was 4 per cent.

(4) The "normal" gallbladders were distributed without significant variation between the cured, improved, and unimproved groups.

(5) Coincident appendectomy or ulcer operation appears to have no influence upon the prospect for cure after the removal of nonpathological gallbladders.

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CLASSIFICATION AND PATHOGENICITY OF CERTAIN MONILIAS*†

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The rôle of monilia in disease has been in controversy since the description of Langenbeck (1839) of the "thrush" fungus. The most common lesions are located on the buccal mucosa and the mucous membranes of the larynx, esophagus, and vagina. M. Gubler⁷ said that they never attack the mucous membrane of the bronchi because of the alkaline secretion. Robins³ made very extensive clinical observations on the nature of the membrane found in cases of thrush and the method by which it is produced. He formulated the theory that the fungus requires an acid medium in which to propagate and that this medium is supplied by acid secretion of the inflamed buccal mucosa. He considered the fungus a harmless parasite adhering to the surface of the epithelium which proliferates and desquamates because of the inflammation. The membrane produced under such conditions is composed of desquamated epithelial cells, tenacious mucus, and tangled masses of mycelium, and is in no sense an inflammatory exudate. Gubler was of the same opinion and said that he never saw the fungus spread into the bronchi where the secretions are alkaline but in a few cases he saw it on the mucosa of the stomach and small intestine. Vogel,²⁴ however, was of the opinion that the thrush fungus sometimes produces true pseudo-membranes such as those seen in cases of diphtheria. These clinical investigators made no distinction between the different species of monilia that are associated with thrush.

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Herf,¹ reported twenty-six cases of vaginitis due to monilia. He stated that the organisms produced severe inflammatory reactions which were associated with burning and itching. Most of his cases occurred during the course of pregnancy. He made cultural studies and identified two types of monilia, *M. albicans* and *M. candida*.

Plaut¹⁵ studied *M. albicans* and *M. candida* in an effort to find distinguishing characteristics for the separation of the "thrush" fungi, *M. albicans*, from *M. candida*, isolated from decaying wood by Bonorden.² These experiments included studies on the pathogenicity of these two species. He used young chickens, pigeons and rabbits. Two methods of inoculation were used for the fowls, by streaking cultures over the mucosa of the beak and crop, and by sewing together a wound made in the crop by a scalpel with silk threads which had previously been soaked in a suspension of these organisms. The young fowls died within four or five days but older ones withstood the infection and recovered after several weeks. He failed to produce lesions in dogs by any method and was only rarely successful when he inoculated rabbits intravenously. He concluded that the thrush fungus and *M. candida* are identical.

Craik,¹ isolated both *M. albicans* and *M. candida* from cases of thrush.

Castellani's^{3,4} report of the frequency of broncho-moniliasis in the tropics created widespread interest. He recognized the infection as manifesting varying degrees of severity and described the disease as being mild, severe and very severe. His studies were confined to the cultural reactions of the organisms isolated from sputum. He reported no postmortem examinations and no animal experimental work.

Ashford¹ isolated cultures of monilia from cases of sprue and called the organism *M. psilosis*. He was able to produce generalized moniliasis in rabbits and guinea-pigs by intravenous injections and was able to exalt the virulence of the cultures by repeated passage through animals. When fed to guinea-pigs organisms whose virulence had been increased by animal passage produced stomatitis and diarrhea.

The literature contains many case reports associating various species of monilia with bronchitis, pneumonia, asthma, vaginitis, and skin lesions. From the tropics Sen¹⁷, Paramanand,¹² and Pijper¹³ have contributed to this literature and in this country Simon,¹⁹ Steinfield,²⁰ Johns,⁹ Shaw,¹⁸ Plass,¹⁴ Gilbert⁶ and others. Their reports are, however, not accompanied by animal experimentation except in a few instances and we have seen no post-mortem reports. The organism isolated have been grouped according to Castellani's classification or have been given new names.

One of us²³ reported eighteen cases of bronchomycosis, recurring attacks of pneumonia going on to bronchiectasis, and varying grades of bronchitis, associated with sputum laden with monilia fungi. In this report no attempt to classify the organisms was made. The cases were divided clinically into very mild, moderately severe and severe infections. One patient in the group died but postmortem was refused.

Nye, Zerfan and Cornwell¹¹ working with cultures isolated from the stools of apparently normal people and from others afflicted with various diseases killed rabbits by intravenous inoculations with cultures classified as *Parasaccharomyces A*. All other types of *parasaccharomyces* (monilia) they found to be non-pathogenic for rabbits even in very large doses. They concluded that following intravenous inoculation of large doses, *Parasaccharomyces A* is mildly pathogenic for rabbits and that occasional human infections may have occurred.

Stovall and Bubolz²¹ classified monilia isolated from sputum in cases of bronchitis, pneumonia and asthma, and showed that the organism isolated could be classified into three species by fermentations and colony formation on malt agar. They²² reported a further study of organisms isolated from sputum and also cultures secured from the American Type Culture Collection. They found that all of these cultures could be classified into three species on the basis of fermentations, reaction in calcium lactate milk and colonies on malt agar (table 1).

It is apparent from this very brief review of the literature that the notion concerning the pathogenic qualities of the various spe-

cies of monilia is vague and that the conflicting reports are in all probability due to the failure of the investigators to recognize the different species.

In view of the conflicting opinions found in the literature and since we were able to classify more than 150 organisms associated with various diseases into three species, Type I, Type II, Type III, we decided to determine the reaction of animals to each of the three types with the idea that further distinctive features might be revealed by characteristic animal reactions for each species and that the reactions would reveal the nature of the disease process.

TABLE 1

DIFFERENTIAL CHARACTERISTICS OF THE THREE TYPES OF MONILIA

| | MALTOSE | SACCHAROSE | MILE | MYCELIAL COLONIES MALT AGAR (48 HOURS) |
|------------------------------------|--------------|--------------|-------------|---|
| Type I..... | Acid | Acid | 0 | + |
| Type II (<i>M. albicans</i>).... | Acid and gas | Acid | Coagulation | 0 |
| Type III (<i>M. candida</i>)... | Acid and gas | Acid and gas | 0 | ++ |

MATERIAL AND METHODS

Five cultures of each type were selected for the work. Since we have found in the American Type Culture Collection, organisms already named which typify each of our groups, we have in this paper used the name of the type culture which typifies the group rather than the group number. In group one, typified by *M. parapsilosis*, four of the cultures are from our own collection and one from Johns Hopkins University. The other two groups, typified by *M. albicans* (Type II) and *M. candida* (Type III) contain two strains each from our own collection and three each from the American Type Culture Collection. A complete history of each culture is recorded in table 2.

Cultures were grown on malt agar for twenty-four hours at 37°C. and washed down with sterile normal salt solution. These suspensions were shaken in flasks with glass beads and standardized for the number of fungus cells per cubic centimeter by counts in a hemocytometer. No mycelium was produced on this medium in the incubation period used.

Rabbits, guinea-pigs, white rats and mice were the animals used and the method of inoculation was chosen to suit the purpose of the particular experiment. Thus intravenous injections of measured doses of the three species were given to rabbits in order to determine the lethal and morbidity dose of each by

TABLE 2

| NUMBER | SOURCE | CLINICAL DESCRIPTION OF CASE AND REMARKS |
|-------------------------------|-----------------------|---|
| Type I <i>M. parapsilosis</i> | | |
| 35221 | Sputum | Mild but persistent bronchitis. Sputum repeatedly negative for tubercle bacilli. Wassermann negative. No further report |
| 35746 | Sputum | Male, age 40, farmer; lost 15 pounds weight; sick three years; two ounces of sputum in 24 hours. Wassermann negative; sputum negative for tubercle bacilli. Died. No autopsy |
| M. parapsilosis 50858 | Sputum | Furnished by J. H. Lamb, Johns Hopkins University, Department of Pathology |
| 36255 | Sputum | Male, age 36, a laborer. Four months before pulmonary symptoms had ringworm on cheek; sick two years; x-ray diagnosis: interlober empyema or abscess. Reported year later blastomycosis from another laboratory. Sputum negative; Wassermann Negative. Recovered |
| | | Chronic bronchitis. No response to inquiry about patient |
| Type II <i>M. albicans</i> | | |
| 33691 | Sputum | Male, age 45, sick two months; sputum negative for tubercle bacilli; severe cough; large ulcer on soft palate, upper and lower lip; fungus in sputum, and fluid from ulcers on lips; dullness over lower right chest posteriorly and anteriorly. Died. No autopsy |
| 22353 | Sputum | Male, age 35, mild bronchitis with elevation of temperature for two months. Recovered |
| 4135 | American Type Culture | <i>M. pseudo-tropicalis</i> Castellani |
| 2112 | American Type Culture | <i>M. albicans</i> ; Natl. Coll. Type Cul., Lister Institute, 714. Isolated by Craik from case of thrush |
| 2117 | American Type Culture | <i>M. psilosis</i> Ashford. Natl. Coll. Type Culture Lister Inst. From J. T. Duncan, London School for Trop. Medicine. Isolated from feces of acute case of sprue |
| Type III <i>M. candida</i> | | |
| 23669 | Sputum | No record of patient |
| 14999 | Buccal mucosa | Membrane covered the mucosa of the cheek, gums and lips to the edge of the skin. Thrush. Patient anemic; membrane present one year |
| 2113 | American Type Culture | <i>M. candida</i> Bonorden. Natl. Coll. Type cultures; Lister Institute, 922, Tanner Collection |
| 1369 | American Type Culture | C. Neuberg, Berlin, Germany (Bonorden Handb. p. 76, Fig. 86, 1857) Thom and Church collection, 4472-2 |
| 750 | American Type Culture | <i>M. tropicalis</i> Castellani; Aldo Castellani, Tulane Univ., isolated 1909 |

this method. In order to test the tissue invasiveness, local injections were made beneath the skin, into various viscera, into the nasal sinus, and into muscles.

In this paper it is not feasible to give in detail all of the experiments that were carried out or to include the results obtained with guinea-pigs, white rats or mice. That will be reported elsewhere. We have, therefore, set forth in this paper the outstanding features of the results of the studies with rabbits.

TABLE 3
DOSE DIFFERENCES OF MONILIA SPECIES
(Dose in millions per 100 grams body weight)

| SPECIES | LETHAL DOSE | MORBIDITY DOSE |
|---|-------------|----------------|
| | millions | millions |
| <i>Monilia parapsilosis</i> , Type I..... | None | 200* |
| <i>Monilia albicans</i> , Type II..... | 1.5 | 0.5 |
| <i>Monilia candida</i> , Type III..... | 35.0 | 6.0 |

Dose variability of Type II

| STRAIN | LETHAL DOSE | MORBIDITY DOSE |
|--------|-------------|----------------|
| | millions | millions |
| 4135 | 1.5 | 0.5 |
| 2112 | 1.5 | 0.5 |
| 22353 | 1.5 | 0.5 |
| 2117 | 3 0 | 1.0 |
| 33691 | 6.0 | 2.5 |

Dose constancy of Type III

| STRAIN | LETHAL DOSE | MORBIDITY DOSE |
|--------|-------------|----------------|
| | millions | millions |
| 2113 | 30 0 | 5.5 |
| 1369 | 25 0 | 5.0 |
| 750 | 30 0 | 5.5 |
| 23669 | 35 0 | 6.0 |
| 14099 | 35.0 | 6.0 |

* No lesions.

DOSAGE

In connection with previous work we had observed that some rabbits died within several hours or days following intravenous inoculations and others tolerated approximately the same dose without ill effect. We did not then know the species of organism used. The average lethal and morbidity intravenous dose for each of the three species and for each of the strains within the species is shown in table 3.

M. parapsilosis demonstrated no pathogenicity in these experiments. We ran the doses extremely high in attempts to establish the tolerance of the animal. The largest dose administered was three billion organisms suspended in 10 cc. of salt solution to a rabbit weighing 1830 grams. The rabbit showed nothing more than a slight elevation of temperature and leukocyte count for twenty-four hours. Postmortem examination of animals injected with the five cultures in this species revealed no lesions.

These results show a wide variability in the lethal and morbidity doses for these three species of monilia, the variation being so distinct for each that the identity of the organism can be predicted on the basis of intravenous dose required to kill a normal healthy rabbit. Thus it is seen that *M. parapsilosis* causes no change in the behavior of rabbits and no lesions at postmortem in doses of three thousand million. *M. albicans* on the other hand killed regularly in doses varying from twenty-five to seventy-five million and morbidity doses varying from ten to fifty million produced acute illness, and extensive lesions in various organs with eventual recovery or death of the animal. *M. candida* shows a sharp contrast to the other two species. It produces death if very large doses are used, six hundred million to eight hundred million, and a few chronic lesions in doses of two hundred to three hundred million. Thus contrasting *M. candida* with *M. albicans* it was necessary to inject five to fifteen times more cells of the former than the latter to cause death or to produce lesions without causing death.

CLINICAL COURSE

In the clinical course the differentiation between the three species was clearly evident and the more virulent effects of *M. albicans* were demonstrated. No rabbits manifested more than a slight rise of temperature and leukocytes for twenty-four hours when inoculated with even massive doses of *M. parapsilosis*. Those receiving relatively small doses of *M. albicans* became very ill, lost weight rapidly and died in eighteen hours to eight days. Death was often preceded by suppression of urine and convulsive seizures. Those to which sublethal doses were administered became very ill

for the following five to eight days and then began to recover but most of them never regained their normal weight. The temperature rose from 103° to 105° and remained up for several days to a week and gradually declined to normal. The leukocyte count revealed the overwhelming effect of this organism. For twenty-four to forty-eight hours following the inoculation there was a marked leukopenia. Unless the animal went into uremia and died the count began to rise and the next day or two a marked leukocytosis appeared and lasted for two weeks or longer. Weight loss and emaciation was marked and all the animals except the ones inoculated with culture No. 33691 became uremic. The evidence of uremia was based on suppression or decreased quantity of urine, elevated non-protein nitrogen and muscle twitching or convulsions. One rabbit showed 260 mgm. of non-protein nitrogen per 100 cc. of blood on the sixth day following inoculation and died. Another rabbit developed a non-protein nitrogen blood retention of 200 mgm. by the sixth day when there were signs of uremia but by the eleventh day the non-protein nitrogen had fallen to 67 mgm. and the animal recovered.

M. candida produced a very much less stormy course. Organisms of this species resulted in a rise of temperature for a few days in no way different from that described for *M. albicans*. The leukocytosis was milder and leukopenia did not occur. The urinary output remained normal; only traces of albumen were encountered and the microscopic examination revealed only occasional leukocytes and casts. Fungus cells were not infrequent but no mycelium was found as it was in animals inoculated with *M. albicans*. The non-protein nitrogen of the blood was not elevated.

GROSS AND MICROSCOPIC EXAMINATION OF TISSUES

It is to be expected that postmortem examination following such widely varying clinical courses would reveal marked differences in tissue reactions. *M. parapsilosis* produced no gross or microscopic tissue changes. Sections of the kidney, lung and liver removed from animals killed within two to twenty-four hours following inoculation showed fungus cells but no mycelium and no budding forms and there was no cellular reaction in the tissue.



FIG. 1

a. Large white kidney of rabbit 23 inoculated intravenously with 52 million cells of *Monilia albicans* (Type II) and killed 6 days later. Non protein nitrogen 200 mgm. per cent. Kidney two and one-half times larger than normal. Thick, anemic cortex with miliary abscesses sharply demarcated from congested medulla.

b. A: *M. albicans* infected kidney; weight of rabbit 1585 grams, dose 62 million, killed on fifth day. C: *M. candida* infected kidney; weight of rabbit 1665 grams, dose 229 million, killed on ninth day.

c. Lungs of rabbits inoculated intravenously with the three types of monilia. P: *M. parapsilosis*; dose 3,000 million. Very rare petechial hemorrhage. A: *M. albicans*; dose 60 million. Numerous petechial hemorrhages. C: *M. candida*; dose 275 million. Few petechial hemorrhages.

d. Gall bladder lesions showing budding and elongated *M. albicans*. Rabbit inoculated intravenously.

e. Budding *M. albicans* in alveolar wall of a rabbit's lung.

f. *M. albicans* in a brain abscess. Mycelia; lateral and terminal conidia.

M. albicans showed characteristic reaction in the lungs and kidneys. The liver, however, proved resistant, rarely showing gross lesions although the gall bladder bile contained abundant mycelium and in some cases abscesses in the wall from which the fungus was isolated (fig. 1, *d*). The kidneys were two to two and a half times normal size, the capsule was tense and the surface appeared very granular due to numerous small greyish-white abscesses pin point to pin head in size (fig. 1, *a*). The cut surface showed a pronounced contrast between the cortex and medulla (fig. 1, *b*). The cortex was greatly thickened, appeared almost white, and was thickly studded with minute abscesses (fig. 2, *a*). The normal cortical markings were absent. The medulla appeared markedly congested and contained a few small abscesses. This description is characteristic for the kidneys of those animals that either died or were killed within the first eight days following the inoculation.

The microscopic examination of sections from these kidneys showed the process to be acute, exudative in type and revealed many fungi in the lesions which appeared not only as single or budding cells but also as strands of mycelia (fig. 2, *c*).

The lungs of rabbits which died or were killed within forty-eight hours after inoculation were characterized by numerous subpleural

FIG. 2

a. Kidney of rabbit inoculated intravenously with 50 million *Monilia albicans* cells and killed two days later. Miliary abscesses in cortex.

b. Kidney of rabbit inoculated intravenously with 330 million *Monilia candida* cells and killed on nineteenth day. Wedge shaped granulomatous areas involving medulla and lower zone of cortex.

c. Mycelia of *M. albicans* in an abscess of the kidney cortex. Modified Gram stain.

d. *M. candida* in kidney lesion. No mycelia formed. Modified Gram stain.

e. Early formation of a mycelium in the intertubular capillary of the kidney. Rabbit killed four hours after inoculation of *M. albicans*.

f. Mycelia with lateral and terminal conidia. Rabbit killed 40 hours after intravenous inoculation of *M. albicans*.

g. Long mycelia in glomerulus and outside of glomerulus. Rabbit died on third day; inoculated with *M. albicans* into the nasal sinuses.

h. Miliary abscesses in skeletal muscle (thigh muscle). Rabbit died on the 4th day after intravenous inoculation of *M. albicans*.

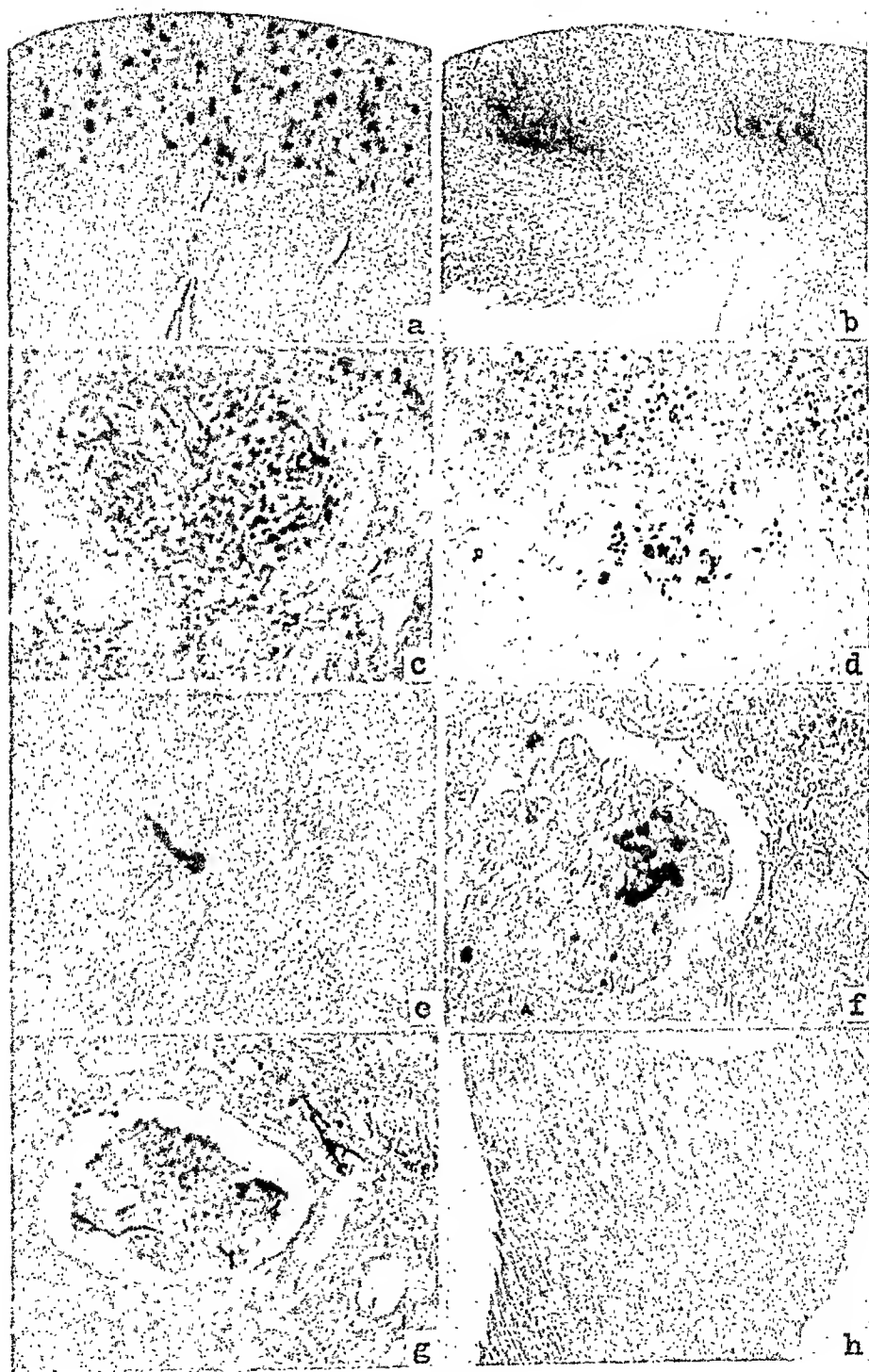


FIG. 2

petechial hemorrhages (fig. 1, *c*), and the cut surface revealed similar hemorrhages ranging in size from a pin point to several millimeters. Thrombosis of the large vessels was seen occasionally; eight per cent of the animals in this group died of pulmonary thrombosis. If the animal lived the petechiae later disappeared and the lung appeared grossly normal.

Microscopic examination of the lungs removed in the first twenty-four hours after inoculation showed a thickening of the alveolar walls. This thickening was not uniform through the alveolar wall but was distributed along the walls like knots in a string. These nodules were capillary thrombi by fungi accompanied by a cellular reaction in the tissue composed of polymorphonuclear leukocytes and mononuclear cells. However, they were not all thrombi; many were cellular reactions around groups of fungi in the tissue. Exudation and hemorrhage into the alveolar spaces caused small, patchy, irregularly distributed consolidations similar to the first stages of bronchopneumonia.

Gram stain demonstrated the presence of budding fungi and mycellia in these lesions (fig. 1, *e*).

Lesions in other tissues throughout the body were frequent. Multiple lesions in the skeletal muscle (fig. 2, *h*), heart muscle and various viscera occurred with the more virulent cultures.

Animals inoculated with *M. candida* showed fewer lesions and fewer structures involved in spite of doses five to fifteen times larger than with *M. albicans*. The kidneys and lungs were the only organs or tissue affected. The kidney, however, was always normal in size, or only slightly enlarged, the capsule stripped with ease, displaying a smooth, pale red surface with an occasional pin point to pin head size greyish white, slightly elevated area (fig. 1, *b*). The cut surface showed normal parenchymal markings. The cortex was not thickened and contained only an occasional wedge-shaped greyish white area which extended through the medulla. There were also other greyish white areas in the medulla.

The microscopic examination revealed an explanation of the striking difference of the kidneys in these animals and those injected with *M. albicans*. The lesions were of a chronic granulo-

matous type, being characterized by infiltrations of lymphocytes, eosinophilic leukocytes, plasma cells, monocytes and a few polymorphonuclear leukocytes. Perivascular lymphocytic infiltrations were not uncommonly seen. The lesion rarely went on to necrosis. Cellular reactions were absent in all rabbits killed two and a half hours to forty-eight hours after inoculation, although fungi were seen in the glomeruli and inter-tubular vessels. The cellular reaction usually did not begin to appear until about the fourth day. They appeared first in the medulla and at this time the lesions were nearly all confined to the medulla. As they increased in size they became wedge shaped and spread to the cortex (fig. 2, b). Mycelium was never present in the lesions or capillaries although budding forms occurred (fig. 2, d).

The gross appearance of the lung was similar to that seen in animals inoculated with *M. albicans* but to a much less degree (fig. 1, c). The pleural surface showed a few subpleural hemorrhages and hemorrhagic areas on the cut surface in those animals on which autopsy was performed two to thirty-six hours after inoculation. The hemorrhagic areas were not present in animals seventy-two hours after injection. None of the rabbits in this group died of pulmonary embolism.

The microscopic examination of sections made from these lungs showed no mycelium in the tissues or associated with lesions but did show occasional budding forms. The alveolar capillaries appeared distended and serum and erythrocytes were seen in the alveolar spaces; eosinophilic leukocytes and lymphocytes and a few polymorphonuclear leukocytes formed aggregations of cells around the arterioles but the nodules seen in animals injected with *M. albicans* were rarely seen and Gram stains showed fungi which had lost their Gram reaction and appeared to be in process of disintegration.

DISCUSSION

The pathogenicity of an organism for one species of animal does not mean pathogenicity for all. And oppositely, the lack of disease producing attributes for one species does not mean non-pathogenicity for all. This has long been recognized and has

resulted in the selection of animals for individual tests. Thus white mice are used for the typing of pneumococci and guinea-pigs for the demonstration of tubercle bacilli; *B. mallei* is highly pathogenic for horses but is harmless for cattle. The results of animal experiments can not, therefore, be used to prove pathogenicity or non-pathogenicity for man, but they are useful in studying the mechanism by which tissue reactions are produced in those animals which react to them and in determining the relative virulence of different species of the same genus and different strains of the same species. Such studies are useful in establishing the identity of species by recognizing a constant difference in pathogenicity for the same animal or a characteristic histo-pathological reaction in the tissues.

In previous papers we have described a method of study by which we were able to classify under three species 150 strains of monilia isolated by us, and Type Cultures which were under a variety of species.

The animal work reported in this paper further emphasizes the significance of these three species. The dose required to kill rabbits, the clinical course of the animals, and the macroscopic and microscopic examination of tissues and organs removed at necropsy of animals injected with representative strains of organisms of each of the three species mark out sharply one from the other. *M. albicans* is revealed as the organism of greatest virulence and *M. parapsilosis* is shown to have no pathogenicity for rabbits, while *M. candida* in overwhelming doses, five to fifteen times greater than *M. albicans*, does produce occasional lesions in the kidney.

These results on the face of the gross and microscopic examinations of the tissue give the impression of a disease produced entirely by thrombosis and that the organism acts only in a mechanical manner by plugging the arterioles. Thus 8 per cent of the animals that died following injections of *M. albicans* died of pulmonary thrombosis, and the characteristic lesions were petechial hemorrhage in the lung and multiple abscess in the cortex of the kidney; all of which indicate a purely mechanical plugging of capillaries and arterioles.

On closer study, however, it is evident that the variability in the pathogenicity of these organisms is due to the difference in their ability to grow in the animal body and to invade the tissue. Microscopic examination of sections of lungs and kidneys removed two and four hours after inoculation shows an early degeneration of *M. parapsilosis* evidenced by its appearance in the capillaries as Gram positive bodies which were beginning to break up. They never produced mycelium in the animal body although it is an abundant mycelium producer in certain culture media. This organism has not manifested any evidence that it has the ability to reproduce in the blood stream. In spite of the fact that it was administered in doses two hundred times larger than the doses of *M. albicans*, it produced no lesions and sections stained with Gram's stain revealed very few fungus cells in the capillaries and many of these were degenerated forms.

On the other hand, *M. albicans* manifested evidence of vigorous and rapid multiplication in the blood stream. The sections of lungs and kidneys, removed at two and four hours (fig. 2, e), showed many Gram positive fungus cells, many budding forms and strands of mycelium in the capillaries of the alveolar walls and glomeruli. As the disease process progressed the mycelium became more abundant (fig. 2, f and g).

By this same kind of evidence *M. candida* showed only feeble powers of multiplication in the blood stream. In sections of lungs and kidneys removed at two and four hours the Gram stain revealed Gram positive fungus cells, a few of which were beginning to lose their Gram reaction.

With this manifestation on the part of *M. albicans* to multiply vigorously in the blood stream, we, of course, expected that while mycelium production would cause mechanical occlusion of some of the arterioles it should also invade tissue. This invasion would be accomplished, if by no other method, by breaking through structures due to rapid increase in the number of fungus cells. With this idea in mind we performed local injections into the nasal accessory sinus, subcutaneous, intramuscular, intrapleural injections and directly into the kidney.

The subcutaneous inoculations resulted in abscesses which

healed in about ten to twelve weeks. The direct inoculation in the left kidney resulted in septicemia with local lesions occurring in the opposite kidney and the brain (figs. 1, b). The intramuscular injections resulted in lesions in the kidneys and the inoculations into the nasal sinuses caused miliary moniliasis. *M. parapsilosis* and *M. candida* produced neither local nor general reactions by this method of administration. These experiments give evidence of the invasive power of *M. albicans* as well as its ability to reproduce in the tissue, and the inability of either *M. parapsilosis* or *M. candida* to invade tissue.

TABLE 4
SIZE OF FUNGUS CELLS IN EACH TYPE

| TYPE | CULTURE NUMBER | LARGEST | SMALLEST | AVERAGE | TOTAL AVERAGE |
|------|-------------------|---------------------|---------------------|---------------------|------------------|
| I | 50858 | <i>micra</i> 7.1 | <i>micra</i> 2.1 | <i>micra</i> 4.5 | 4.63 |
| | 35221 | 7.1 | 2.1 | 4.73 | |
| | 38746 | 6.0 | 3.2 | 4.65 | |
| II | 4135 | 12.0 | 2.8 | 6.1 | 6.03 |
| | 801 | 9.7 | 2.4 | 6.0 | |
| | 33691 | 9.5 | 3.1 | 6.0 | |
| III | 750 | 9.9 | 3.4 | 6.4 | 6.37 |
| | 14999 | 8.7 | 4.2 | 6.43 | |
| | 23669 | 11.0 | 3.0 | 6.29 | |

One criticism that may be offered is that the doses were extremely large and that the conditions are, therefore, highly artificial. This objection may be offered to many animal experimentations. No animal experiments ever reproduce the circumstances and environment of infection in man. That the size of the dose was not the determining factor was illustrated in these experiments by the fact that the organisms which showed no or but little pathogenic properties were the very organisms which were administered in doses of two hundred to a thousand times larger than those which demonstrated these characteristics.

We were further concerned about the mechanical factor involved in the pathogenicity of these organisms. If the results were

due only to mechanical occlusion of arterioles and capillaries, it seemed reasonable that the species which was most constant in its production of lesions when administered in relatively small doses would be much greater in physical size. We, therefore, grew three cultures of each species for forty-eight hours on malt agar and suspended the growth in normal saline. The size of the cells in the suspension was determined by measuring 100 cells in each suspension. Table 4 shows the results.

It is seen from this table that *M. parapsilosis* is the smallest organism and it also is not pathogenic; but, on the other hand, *M. candida*, which shows only feeble ability to grow in the animal body and to produce lesions has an average size somewhat larger than *M. albicans*. The variability in size of the different species does not reveal enough difference to explain their difference in pathogenicity.

To examine still further the idea of plugging of arterioles and capillaries, when the factor of reproduction in the blood stream or tissue was eliminated, we prepared suspensions in normal saline of cultures of *M. albicans* and *M. candida* to which was added enough formaldehyde to make a 0.5 of 1 per cent solution. When the organisms were dead, proven by culture, rabbits were inoculated with doses of each which had previously either killed the animal or produced numerous lesions. No animal showed any sign of illness following these injections and gross and microscopic examination revealed no vascular obstruction.

CONCLUSION

We believe that these experiments have revealed a fundamental difference in pathogenicity for rabbits, for the three species of monilia used in these experiments; that the postmortem examination four hours after inoculation of rabbits given intravenous doses as described in this paper reveals characteristic differences for the three species of monilia used, and that simple mechanical occlusion of arterioles and capillaries does not explain the nature of the pathogenicity, but that ability or failure to grow in the animal body and invade tissue has been demonstrated to be the principal quality accountable for the decided virulence of *M. albicans*,

the feebly pathogenic properties of *M. candida* and the lack of this characteristic in the case of *M. parapsilosis*.

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MONILIASIS OF THE LUNGS AND STOMACH

CASE REPORT WITH AUTOPSY*

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One finds in the literature a good many references to the isolation of monilia or yeast-like organisms from the sputum. A few of the reports describe clinical conditions of either mild or severe pulmonary moniliasis. But undoubtedly in many instances the finding of monilia in the sputum indicates only, that these organisms are at most secondary invaders. Some authors report fatal cases attributed to infection of the lung with monilia.

In 1924, Johns¹ reported five cases of moniliasis, and mentioned the work of Castellani, who, in 1905, described the condition in Ceylon. Johns stated also that only two cases had been reported in this country previous to his own. For complete information concerning the subject, including the historical references in the literature, clinical and experimental data, and data for identification and classification of the monilia, one should consult the following authors in addition to those mentioned above: Stovall and Greely,⁶ Stovall and Bubolz^{3, 4, 5} and Stokes and Kiser.²

A limited survey of the literature does not reveal any necropsy report linking up the clinical diagnosis of pulmonary moniliasis with pathological findings in the lung. Stovall and Greely⁶ mentioned having isolated monilia from autopsy material in one case. Therefore the following case history with pathological data is presented. No attempt is made to enter into a complete description of any phase of the report, especially the cultural or animal studies made with the organism isolated.

* Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9-12, 1933.

CASE REPORT

Mrs. B., aged fifty years, white, with a feeling of well being and "don't care" attitude not in keeping with her physical appearance, only after much prompting, stated that for the past two years she had suffered from "indigestion," cough with expectoration, a pain in the back between the shoulder blades, and loose bowels, two to six movements a day, with exacerbations. In fact it was quite difficult to get her to admit that she was ill in any way.

Physical examination presented a fairly well developed, but very emaciated, white female, with a slightly yellow color, wrinkled skin, atrophic and inelastic tissues. The knee jerks were markedly diminished, pupils were equal and reacted to light. There were no enlarged nodes. She presented glistening sclera, artificial denture, a smooth red, glazed tongue, and the entire pharynx was markedly pale and anemic. The tonsils were atrophic and contained sero-pus. The thyroid was small. The thorax presented bony fixation, hyperresonance in the uppers and considerable impairment of resonance over the left base posteriorly, with diminution in breath sounds, sonorous, sibilant and crepitant râles, generally scattered over the chest but more marked over the bases, particularly the left. The heart was not enlarged; there was no increase in the upper mediastinal dullness, no murmurs, no arrhythmia, and no change in sounds. Her blood pressure was 110 systolic, 70 diastolic. The abdomen tympanitic throughout. The spleen was not palpable. There was considerable tenderness over the region of the gall bladder, liver, and pylorus. Vaginal examination revealed an atrophic uterus, ovaries, and cervix, without other evidence of disease. The rectum presented a number of tags and varicose veins. Extremities showed no disturbance in sensation. There were numerous pigmented scars on both legs, due to ulcers many years before. The temperature ranged from 99° to 102°, pulse from 100 to 130. The patient coughed a great deal and brought up daily several ounces of light greenish, thick, creamy sputum.

Radiographic examination of the chest showed an elevation and partial fixation of the left diaphragm, which was rendered more or less obscure by the pathology at the base of this lung. The cardiac and great vessels were not enlarged; the right pulmonic field presented numerous dilated bronchioles and bronchiectatic changes at the base, while the left pulmonic field showed well defined areas of consolidation in the lower lobe. The upper half of the fields were free from infiltrative processes and showed extensive bronchial dilatation with fibrosis, presenting a general honey-comb appearance over the entire area. The radiologist's conclusions were: Chronic broncho-pneumonia, bronchiectasis, and purulent bronchitis.

The urine showed a few pus cells and erythrocytes, a few hyaline and fine granular casts. The stool was brown, semi-solid, many *Strongyloides* larvae present, no amebae, no monilia in smears; cultures were not made. The gastric contents contained no free HCl even after histamine, but many pus cells, monilia, other organisms and occult blood. Many examinations revealed a thick,

creamy, light green sputum, with a musty odor, and consisting of mucus, elastic tissue, large numbers of monilia, and a few other organisms; no blood was seen. Occasionally the monilia were scarce, or absent in smears, but by incubating the sputum itself, or making cultures, monilia were always found. Often specimens examined immediately after expectoration revealed enormous numbers of monilia. Acid-fast bacilli were never found in the sputum.

Wassermann, Kahn, and slide precipitation tests were negative, and the spinal fluid was normal. Many examinations of the blood revealed a pernicious-like type. On admission: erythrocytes, 2,515,000, hemoglobin, 11.5 grams or 81 per cent, color index, 1.57 volume index, 1.35, reticulocytes, 2 per cent, blood platelets, decreased, moderate anisocytosis and poikilocytosis, slight polychromatophilia and stippling, and a rare nucleated erythrocyte. Van den Berg, direct negative, indirect two units. Slight trace of urobilinogen in urine.

It was therefore concluded that the patient was suffering from pernicious anemia, monilia infection of the lungs and stomach, and a chronically diseased gall bladder and appendix, with degenerative changes in the liver, and a mild nephritis. She was placed on large doses of potassium iodide by mouth, dilute hydrochloric acid, a diet of fresh foods, liver, and liver extract by mouth. Blood transfusion and iron were also tried. With only temporary improvement shown in the midst of her illness, she died after five months of observation and treatment.

Guinea-pigs inoculated with the sputum treated with antiformin never showed any evidence of tuberculous infection. Inoculation of untreated sputum produced local abscesses in guinea-pigs. Most of the pigs inoculated intraperitoneally, intrapleurally, and into the liver, developed monilia lesions. Certain pigs died in three or four days, some in ten to fourteen days, and others survived. The culture is still pathogenic for guinea-pigs, though it does not produce death so quickly as before, or so frequently. The lesions produced are abscesses or pseudo-tubercles, which undergo fibrosis to a great extent. In the lungs of the animal there is produced the same type of reaction.

The organism is oval shaped, from 3 to 10 microns in diameter, is gram positive, and produces rarely a few short branches or hyphae on media. Acid and gas developed in glucose, levulose, mannose, maltose, galactose, and acid only in sucrose. There was no reaction in lactose, inulin, raffinose, dextrin, gelatin, or litmus milk. There were no mycelial colonies on malt agar at the end of forty-eight hours.

The monilia (*albicans*) in question would fall into type II, of Stovall and Bulbolz.

Autopsy report

External examination. The body is that of a white female, aged about fifty-years, and is extremely emaciated. The skin is dark, cyanotic, or brownish colored. The tongue is generally reddened and the papillae atrophic. The anterior surfaces of both legs are covered with large dark pigmented scars or patches.

Internal examination. Abdomen: The little subcutaneous fat remaining is somewhat yellowish. The liver is not enlarged, and presents a nutmeg appearance with much fatty degeneration. The gall bladder is covered with old adhesions, is thick and white, but stones are not present. On handling the stomach and breaking up adhesions it is torn at the pylorus, which is seen to be the site

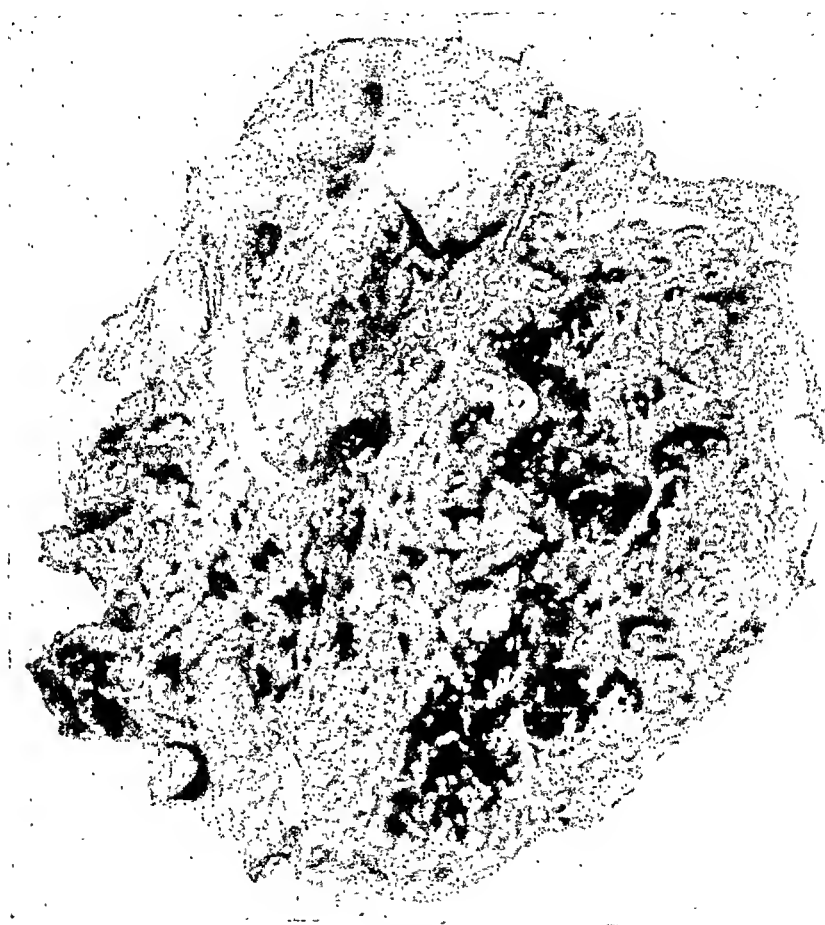


FIG. 1. SMEARS FROM PYLORIC ULCERS SHOWING MONILIA

of a large ulcer which has almost perforated the organ. On opening the stomach the mucosa is found to be eroded and atrophic, and presents numerous petechial hemorrhages. Near the pylorus the mucosa is especially atrophic, and four ulcers are seen, one above and one below the ring, and two at the ring, one of which had been ruptured by handling. These ulcers are filled with a yellowish, greenish, thick, creamy exudate. Smears later revealed many monilia, almost

always without other organisms. The spleen is considerably enlarged, the capsule strips easily. The cut surface is reddish brown, the pulp comes away easily, revealing a good deal of fibrosis. The kidneys are slightly enlarged, capsule strips easily, revealing a reddened surface, the blood vessels in the cortex being very prominent.

Thoracic cavity: There is no fluid present, the pleura is freed from the thoracic cage and diaphragm with great difficulty, and is about 3 mm. in thickness over a great part of both lungs, which show about the same pathology, the left being more involved. The apices are practically free of pathology. On cutting into the lung a remarkable picture is presented, a greater part of both lungs being

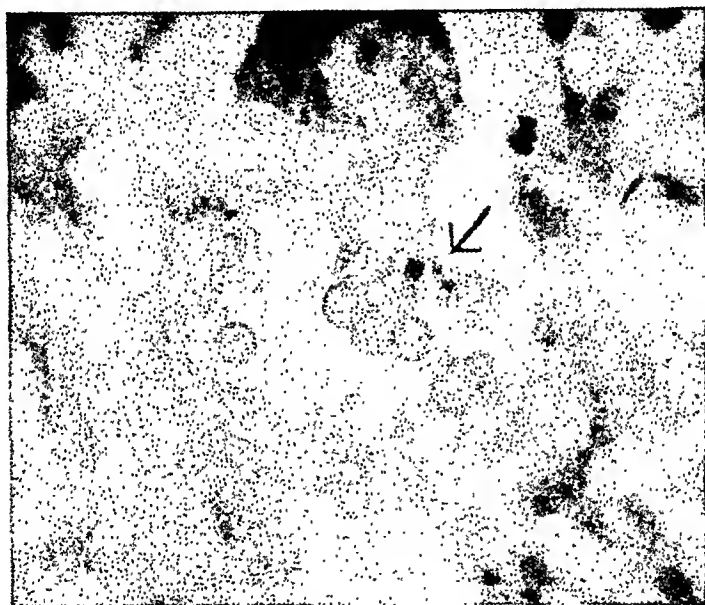


FIG. 2. LUNG OPENED TO SHOW LESIONS

Note multiple cavities throughout associated with bronchial passages. They were filled with greenish pus.

involved. Superficial lung tissue is fairly normal and crepitant. The lung (fig. 1) is honey-combed with countless small pus pockets, ranging from 3 mm. to 2 cm. in diameter. These pus pockets are mostly associated with the medium-sized and small bronchioles, but also involve the adjacent lung tissue. The pus is light greenish in color, smears later revealing monilia in almost a pure state. Very little normal lung tissue remains, as the areas not showing pus are mostly very dense or fibrous. It is difficult to understand how the patient existed so long with so little normal lung tissue.

The heart is not enlarged, the pericardium is slightly thickened, and the myocardium is somewhat brownish and fibrous. There is no evidence of valvula disease. The aorta shows a few arterio-sclerotic plaques.

Bone marrow of the femur shows a red hyperplastic condition grossly. Smears revealed a very active bone marrow, such as is seen in pernicious anemia. Prussian blue reactions were obtained with tissue from the spleen, kidney, and liver.

Microscopic examination of the lungs: There are several types of lesions present, depending upon the stage of the process. (a) Pseudo-tubercles consisting of a central area of pus cells and mononuclear cells, then fibroblasts, epithelioid cells, plasma cells, and young blood vessels; monilia are scattered throughout (fig. 2). There are very few giant-cells and these are not typical. (b) Fibrosis or granulation tissue (fig. 3) in various stages, with aggregations of small round

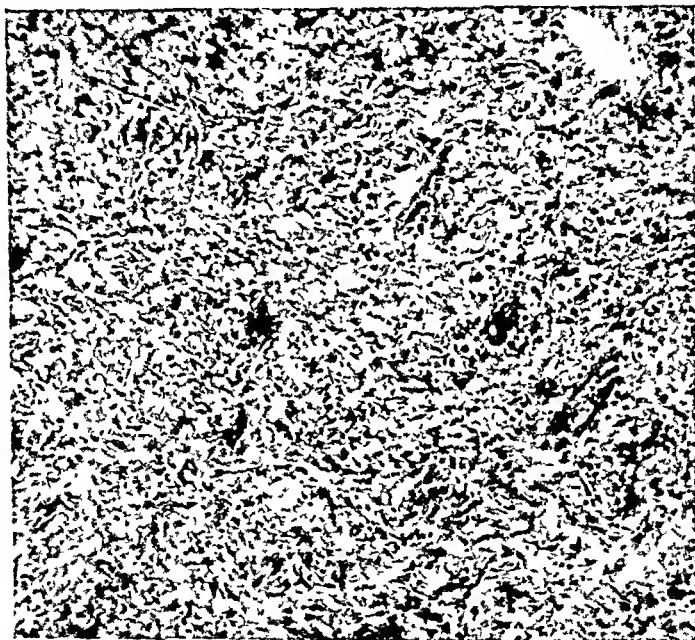


FIG. 3. SECTION OF LUNG

cells and plasma cells. (c) Around the bronchioles of various sizes are chronic inflammatory changes with much fibrosis. Adjacent lung tissue shows a diffuse fibrosis or chronic lobular pneumonitis. Very few areas show lung tissue without chronic inflammatory changes. There is a marked tendency for healing of the pseudo-tubercles by fibrosis, except the bronchiectic areas. Calcification was not found. Areas like that seen in tuberculous infection were not found.

Sections of the stomach ulcers present an ordinary ulcerative type of reaction superficially, and deeper chronic inflammatory changes. Some of these areas present granulation tissue similar almost to sarcoma.

Sections of the liver show a diffuse hepatitis of a fatty and parenchymatous

degenerative nature, with deposits of blood pigment, and some cirrhosis and congestion.

Sections of spleen show passive congestion, pigmentation, and fibrosis.

Sections of the gall bladder show marked chronic cholecystitis.

Sections of the heart show parenchymatous degeneration, fibrosis, and some pigmentation.

COMMENT

The patient presented clinical and pathological evidence of a severe late monilliasis of the lungs and stomach, in addition to the picture of pernicious anemia. The blood picture, absence of free HCl after histamine, and the pathological findings necessitate the diagnosis of pernicious anemia in addition to that of monilliasis, though the slight trace of urobilinogen in the urine, and only moderate increase in the van den Berg reaction is not the usual finding in pernicious anemia. Then too, the condition of the patient's blood did not improve a great deal under adequate liver therapy. This might be explained by the severe accompanying monilliasis, and perhaps lack of a potent liver extract to administer. On the other hand the pernicious-like anemia might be attributed to monilliasis of the lungs and stomach.

It would have been interesting to have made roentgenograms later in the disease to show progress of the lesions. Intravenous iodides, copper, gentian violet, or autogenous vaccines might have been tried, but on the whole, I believe the disease was too far advanced for any method of treatment to have benefited the patient. No agglutination tests with the patient's or with animal's serum were made. Blood cultures were not made.

SUMMARY

(1) A fatal case of pulmonary and gastric (pyloric) monilliasis accompanying a pernicious-like anemia is presented with fairly complete antemortem and postmortem data.

(2) The patient presented clinically a picture similar to that of advanced pulmonary tuberculosis.

(3) The copious sputum contained large numbers of *Monilia albicans*, type II (Stovall). The monilia isolated is rather pathogenic for guinea pigs (other animals not used), even after more than two years of artificial cultivation.

(4) Iodides by mouth did not stop the progress of the disease, perhaps due to the late extensive involvement when treatment was begun.

(5) More frequent study of sputa is indicated for the purpose of detecting monilia and other fungi infections of the lungs.

(6) Monilia in sputum is a rather frequent finding, and is perhaps more often a nonpathogenic secondary invader.

(7) The finding of monilia should not prevent a further search for other causes of pulmonary pathology.

The author wishes to acknowledge the aid and coöperation rendered by Drs. H. J. Mixson, F. Y. Durrance, and J. D. Blevins and Carolyn Van Zandt, the latter superintendent of the Jefferson County Tuberculosis Hospital. Also I am indebted to Dr. W. D. Stovall for his cultural studies in confirming my exact identification of the monilia organism described.

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NEW METHOD OF RETICULOCYTE ENUMERATION

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A new method of reticulocyte enumeration is proposed on the premise that it is a rapid and accurate procedure.

The staining solutions and technique employed in making the blood preparation for study are as follows:

Solution A

| | |
|--------------------------------|-----------|
| Neutral potassium oxalate..... | 1.0 gram |
| Sodium chloride C.P..... | 0.85 gram |
| Distilled water..... | 100 cc. |

Solution B

| | |
|-------------------------------------|-----------|
| Brilliant cresyl blue..... | 1.0 gram |
| Sodium chloride C.P..... | 0.85 gram |
| Distilled water..... | 100 cc. |
| Chloretone (as a preservative)..... | 1.0 gram |

In a conical tipped centrifuge tube place 25 parts of solution A and 5 parts of solution B, mix,* and add several drops of blood. After thoroughly mixing, permit to stand 10 to 20 minutes, then centrifugalize for 20 to 30 seconds at a moderate speed. The supernatant fluid is pipetted off until a layer of fluid approximately equal to the depth of the sediment remains. Mix the sediment well with the supernatant liquid, draw up in the pipette and discharge one drop near the end of a perfectly clean glass slide.

Spread the stained drop with the edge of a coverslip square 18 mm. and draw the film to about 6 cm. from the starting point. The characteristic graded film prepared in this way appears as illustrated in figure 1 when dry and ready for study. The underlying principle of the method involves the Relative Reticulocyte Distribution (R.R.D.) throughout the smear. To know the relative distribution of reticulocytes is important, since it is technically not possible to make a blood film manually so that the distribution of erythrocytes and reticulocytes is equal throughout the preparation. It has been determined, however, that the distribution varies in a definite way when the above technic of preparing smears is followed each time.

* Since a precipitate forms when the solutions are mixed, it is better to make up a larger volume, filter and place the proper amount of the mixed solution in the tube and proceed.

Technic of reticulocyte count

The R.R.D. is found as follows: Divide a piece of paper into 10 columns and mark them 1 to 10. Adjust a 4x eyepiece to a tube-length of 190 mm., using an oil immersion objective 1.8 mm. \times 95 N.A. 1.30 in order to obtain the specific microscopic working field. Any other combination giving the same field may be used.

The R.R.D. in the film will usually be found to show about four variations in concentrations as follows: (1) extremely high, (2) high, (3) low, (4) extremely low or absent.

Place the objective on the upper edge of the film near the starting point, that is the extreme left border of the film (See fig. 1). Moving the slide upwards in a vertical line, count several fields that show the highest as well as the moderately high, moderately low and low reticulocyte concentrations. Record the counts of each classification in column 1. When the lower edge of the film is reached, move the slide to the left horizontally 0.5 cm. (measured by the mechanical stage) from this point move the slide downward, count the reticulocyte concentrations as before and record them in column 2. Count ten vertical zones in this manner. If a vertical zone does not show any reticulocyte, mark in the corresponding column a zero.

In a vertical zone 18 mm. in length there will be found approximately 150 microscopic fields, the average field showing about forty erythrocytes. There should be counted and recorded in each column the number of reticulocyte concentrations found in at least eight to ten fields in each vertical 18 mm. zone. Select (for example see table 1), from the 80 to 100 individual enumerations recorded in the ten columns ten reticulocyte counts distributed as follows:

- (a) Two fields showing extremely high concentrations.
- (b) Three fields showing high concentrations.
- (c) Two fields showing low concentrations.
- (d) Three fields showing extremely low to absent concentrations.*

Having added the ten reticulocyte counts the total number is referred to as the sum of reticulocytes (S_R). In the same ten fields there are 400 erythrocytes. The ratio of reticulocytes to erythrocytes (for example see table 2) is therefore,

$$\frac{S_R}{400}$$

The percentage of reticulocytes is

$$\frac{S_R}{400} \times 100$$

or

$$\text{per cent} = S_R \times .25$$

* When selecting the individual reticulocyte counts use as many zeros as there have been zero columns. (See table 2.)

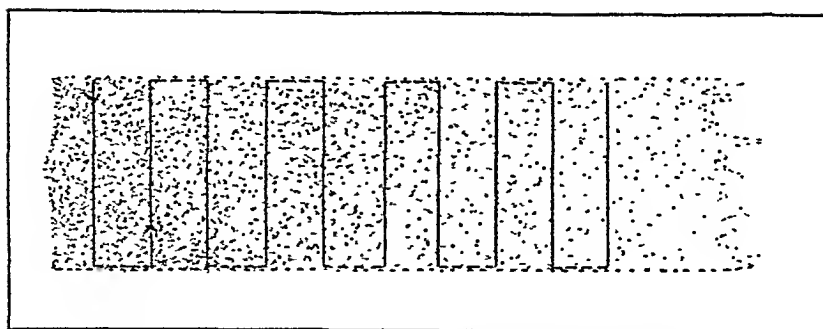


FIG. 1. METHOD OF COUNTING RETICULOCYTES

TABLE 1
EXAMPLE OF METHOD OF RECORDING RETICULOCYTE COUNTS

| COLUMNS | | | | | | | | | |
|---------|----|----|----|----|----|---|---|---|----|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 12 | 10 | 10 | 9 | 6 | 4 | 4 | 7 | 2 | 2 |
| 7 | 15 | 12 | 6 | 5 | 5 | 2 | 8 | 1 | 2 |
| 9 | 10 | 6 | 6 | 10 | 8 | 4 | 3 | 2 | 3 |
| 18 | 9 | 12 | 8 | 5 | 12 | 6 | 8 | 4 | 1 |
| 12 | 6 | 5 | 8 | 3 | 6 | 4 | 5 | 3 | 1 |
| 12 | 6 | 14 | 4 | 5 | 4 | 6 | 5 | 4 | 1 |
| 16 | 12 | 10 | 10 | 4 | 4 | 8 | 7 | 5 | 2 |
| 7 | 14 | 8 | 10 | 7 | 4 | 5 | 4 | 4 | 1 |
| 15 | 8 | 7 | 4 | 10 | 4 | 6 | 3 | 5 | 3 |
| 9 | 4 | 9 | 9 | 6 | 5 | 4 | 2 | 2 | 2 |

TABLE 2
EXAMPLES OF CALCULATING RESULTS OF RETICULOCYTE COUNTS

| A* | B | C | D |
|--|---|--|---|
| 18 | 8 | 2 | 3 |
| 16 | 5 | 2 | 2 |
| 15 | 4 | 1 | 1 |
| 14 | 3 | 1 | 1 |
| 10 | 3 | 1 | 1 |
| 8 | 2 | 1 | 1 |
| 6 | 2 | 0 | 1 |
| 4 | 1 | 0 | 0 |
| 2 | 1 | 0 | 0 |
| 1 | 1 | 0 | 0 |
| $S_R - 94 \times .25 =$ 23.2 per cent | $S_R - 30 \times .25 =$ 7.5 per cent | $S_R - 8 \times .25 =$ 2.0 per cent | $S_R - 10 \times .25 =$ 2.5 per cent |

* The counts recorded in this column are taken from table 1, columns 1, 2, 4, 7, 9 and 10 in accordance with the method described above for selecting individual reticulocyte counts on the basis of relative reticulocyte distribution.

COMMENT

This method of calculating a reticulocyte percentage is rapid and accurate. The method eliminates the enumeration of erythrocytes. It requires only ordinary apparatus and any observer recounting the same film is able to arrive at practically the same result on each enumeration. The limits of error of the method herein described are ± 1 per cent, showing a much lower technical error than other methods. Low concentrations as well as high concentrations of reticulocytes are readily calculated.

From the careful studies of several hundred smears made from normal human blood showing balanced red blood cell and hemoglobin concentrations by this method, I have established that the normal range of reticulocyte concentration is 2 to 3 per cent, with an average of 2.5 per cent.

The author acknowledges with thanks the interest shown by Dr. E. A. Sharp, Director of the Department of Experimental Medicine, and Dr. E. P. Bugbee, Research Physiologist, Parke, Davis and Company.

A CULTURE MEDIUM FOR RAPID GROWTH OF *PASTEURILLA TULARENSIS*

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A pressing need for large amounts of dense suspensions of *P. tularensis* has resulted in the formulation of a new modification of Francis'¹ original cystine-dextrose agar. A medium was wanted that could be prepared quickly and that would support a rapid and abundant growth of this fastidious and rather delicately growing organism. After many trials the present medium was adopted as the most satisfactory one in these respects, and during the past two years its routine use has simplified the matter of obtaining a large quantity of antigen.

PREPARATION OF THE MEDIUM

Twenty-four grams of "Difco" dehydrated brain-veal agar and 2 grams of nutrose are dissolved with gentle heat in one liter of distilled water containing 5 grams NaCl, 0.3 gram Na_2HPO_4 , 0.2 gram KCl and 0.1 gram CaCl_2 . The mixture is divided equally among four 500 cc. flasks and set in the refrigerator to solidify. After solidification 0.1 gram of cystine is placed in a small pile on the center of each surface. If care is taken not to stir the cystine around the medium may be autoclaved safely at fifteen pounds for fifteen minutes without impairing the growth-promoting property of the cystine. Upon removal from the autoclave the flasks are set in a row and each is rotated vigorously in turn until the sedimented cystine is dissolved. This usually requires about ten minutes.

If only 250 cc. of enriched medium is desired, three flasks are capped and put in cold storage and one is cooled to about 50°C. To this flask is added 10 cc. of 25 per cent dextrose and 30 cc. of either ascitic fluid or any sterile serum diluted 1:5. The contents are well mixed, then tubed and slanted or used to fill Blake bottles. Other flasks are melted and enriched similarly as needed. Slanted tubes are capped as soon as set to prevent water loss. If a liter of finished medium is wanted the base medium may be solidified in one large flask, 0.35 gram of cystine added, autoclaved, cooled and enriched with 40 cc. of 25 per cent dextrose and 120 cc. of ascitic fluid or diluted serum.

COMMENT

The medium is characterized by a low agar content with a rubbery, resilient surface which facilitates rapid inoculations and washing and minimizes the chances for ploughing up medium into the bacterial suspensions. This combination of low agar content, maximum hydration with but little free water, and a good working surface is made possible by the inclusion of nutrose.

P. tularensis will grow rapidly and luxuriantly on the surface but not in the water of syneresis. The seventy-two hour growth from three Blake bottles will yield about 50 cc. of a washed suspension of 5,000 turbidity. Growth on slants is best at partial oxygen tension and, because of the tendency of the slants to slump when warmed, they are incubated in a slanting position. Partial oxygen tension is obtained by the tandem arrangement devised by Wherry and Oliver.² Although *P. tularensis* is described as aerobic it is more properly a microaerophilic or partial tension organism. On all mediums on which I have been able to grow it the growth has invariably been faster and more abundant at partial oxygen tension than under aerobic conditions.

The medium is not good for preservation. Cultures will usually not remain viable, as judged by subculturing, for more than eight days. Strains that are being carried along on it are best transferred twice a week. For isolation of *P. tularensis* from infected rodent tissues it is only fairly good, inferior to blood-cystine-dextrose agar and to coagulated egg yolk. Its chief virtues are its capacity to promote rapid and abundant growth, and the short time and ease of making. The base medium can be stored indefinitely at low temperature, and tubes of the enriched medium are good for at least three months if water loss is prevented.

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THREE NOTES ON BIOLOGICAL STAINS

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1. COMBINED PEROXIDASE-WRIGHT'S STAIN FOR BLOOD FILMS

I have been using since 1928 a combined peroxidase-Wright's stain procedure which seems technically superior to Washburn's² method in that the basic fuchsin has been omitted from the oxydase reagent, eliminating the decolorizing step of his procedure, and in that prolonged staining periods are not required. It is not necessary to stain the film within a few hours after its preparation. I have obtained perfect stains on films which have stood as long as forty-eight hours at room temperature.

The oxydase reagent consists of 0.3 gram of pure benzedine base dissolved in 100 cc. of 95 per cent ethyl alcohol to which is subsequently added 1.0 cc. of a saturated aqueous solution of sodium nitroprusside. It keeps well, but should be made up freshly about once every six or eight months. The blood film is flooded with this reagent which is allowed to act for one minute.

The secret of success in peroxidasing the film lies in securing the proper concentration of hydrogen dioxide in the subsequent treatment. The traditional concentration of 1:200 made by adding three drops of a 3 per cent hydrogen peroxide solution to 15 cc. of water is probably nearly perfect, but the instability of the commercial reagents renders it difficult to be certain of exactly this proportion. In preparing to peroxidase a leukemic blood I run a number of films from normal controls through a series of four or five different hydrogen peroxide dilutions, using the standardized time factors given below, in order to determine which is most suitable. If this is done it will be found that as the concentration of hydrogen dioxide increases the jet black peroxidase granules in the finished stain first become more minute and discreet and subsequently turn to a greenish brown in color, finally

from too strong peroxide concentrations disappearing altogether, due apparently to the bleaching action of the reagent. This fact can be further demonstrated by dropping a strong peroxide dilution upon the center of the film flooded with the oxydase reagent, allowing it to spread to the edges by diffusion, when it will be found that the most perfectly stained granulocytes lie on the edges of the film, those in the center showing the greenish brown coloration. When the proper dilution of hydrogen peroxide is determined, without pouring off the oxydase reagent, add a little less than half of the amount of this reagent used. Drop the peroxide dilution onto the flooded film at the ends of the slide and endeavor to secure uniform diffusion by blowing on the surface of the liquids or tilting the slide slightly back and forth. Let the combined reagents act for not longer than two minutes.

The success of the counterstain by Wright's stain upon the peroxidased film depends upon controlling the ion concentrations. The peroxidasing procedure tends toward acidifying the film which results in accentuated reds by Wright's technic and depression of the blue staining. The film is therefore best washed thoroughly after pouring off the oxydasing solution in running tap water which is slightly alkaline (pH 8.0-8.2) for a full minute and allowed to dry in air. The drying may be hastened by blotting or under an electric fan, the latter being preferable.

The film is next stained by Wright's method in the usual way. All bloods after the peroxidasing treatment require slightly longer staining periods but I have not found that leucemic bloods require any longer staining than normals.

The time factors for the various steps which I have found most suitable are: for peroxidasing, one minute by oxydase reagent followed by two minutes after the addition of the hydrogen dioxide solution. For Wright's staining after washing and drying, three minutes by the stain followed by five minutes after the addition of the water.

2. AN ORANGE-BROWN GRAM COUNTERSTAIN

Basic fuchsin and safranin are unquestionably the most practical aniline dyes for use as Gram counterstains in a routine

laboratory, due to the ease of preparation of the stain from stock solutions which keep indefinitely. Where large numbers of slides are being examined continuously, as in the microscopic examination of routine throat cultures made on every patient admitted to a hospital, the red color of Gram negative organisms eventually becomes tiring to the eyes. Bismarck brown, which was at one time strongly recommended as a counterstain and then fell into disuse through the instability of its solutions, has come to the fore again since Huntoon¹ has shown how to make staining reagents of good keeping qualities from this dye by the inclusion of 25 to 30 per cent of glycerine in the formulas. Bismarck brown gives a rather dark brown to Gram negative organisms which often does not contrast well with the Gram positive color. The following formula has been found to keep indefinitely and to give a rich orange-brown color that is both more restful to the eyes than fuchsin or safranin alone and contrasts strongly with the Gram positive blue:

Shake up 1 gram of Bismarck brown in a flask with 100 cc. of 25 per cent aqueous glycerine. Add 1 cc. of stock saturated alcoholic basic fuchsin or safranin and let stand with occasional shaking at room temperature for a day or two. Filter into the dropping bottle.

3. A STABLE PAPPENHEIM PYRONIN-METHYL GREEN STAIN

Two new dyes have recently been placed on the market by National Analine and Chemical Co., Inc., which they have developed in co-operation with S. A. Scudder² and the Commission on Standardization of Biological Stains, especially for use in Pappenheim's stain. They are known as pyronin, "yellowish," which corresponds more closely to the pyronin "gelb," of German manufacture than any other heretofore produced in this country, and methyl green-national, which is an over ethylated product especially recommended for use in the Pappenheim stain. In ordering the latter it is necessary to specify certification number subsequent to NG 7, with which lot the new formula went into routine production.

For those who prefer the color differentiation of the Pappenheim stain either as a histologic stain or as a Gram counterstain these

new dyes will represent a distinct advance. At the time of presentation of her paper Scudder had not found any means of stabilizing the Pappenheim formula so as to avoid the necessity of making up a fresh lot of stain each time that it was desired to use it. I have tried the effect of 25 per cent of glycerine as suggested by Huntoon's work and found it entirely successful. Made up in this way the stain has remained apparently unaltered in its staining characteristics for over seven months. It may be prepared according to Scudder's formula:

| | |
|---|----------|
| Pyronin, yellowish..... | 0.1 gm. |
| Overethylated methyl green..... | 1.25 gm. |
| Hot 25 per cent glycerine in distilled water..... | 99 cc. |

Or two stock solutions of 2 per cent methyl green and 0.3 per cent pyronin, yellowish, can be made up in 25 per cent glycerine and mixed, in various proportions, according to the individual taste, light, and character of work.

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EDITORIAL

THE IMPORTANCE OF CELSUS TO MODERN MEDICINE AND PATHOLOGY

It will be a source of satisfaction to physicians generally to know that the Loeb Classical Library has in preparation a new English translation of the Eight Books of Celsus on Medicine. During the century from 1750 to 1850 it was the practice abroad "to prescribe Celsus as one of the tests by which the candidate is to be tried." Alex. Lee in the preface to his admirable English translation of 1831 gives the reason why Celsus should be taught in all medical curricula:

Even where our author is evidently wrong, for instance in his anatomical descriptions, I have preferred to err with him, rather than be right against him: for this very reason, that the student may see Celsus as the faithful representative of medical science in his age, and contrast that with the present improvements.

As Aurelius Cornelius Celsus lived from 25 B.C. to 50 A.D., he was contemporary with the beginning of the Christian era. If Celsus had been taught continuously, our medical students would always have had a "point of departure" with which to compare current medicine.

If medical students from the time of Thomas Sydenham had been required to know the medicine of Augustan Rome, there would not have been so many discoveries in therapeutics, dietetics, internal medicine and surgery as has been the case, for Celsus was well aware of many of the things in medicine and pathology which we attribute to a very much later age. Largely because he wrote books on other subjects as, for instance, agriculture, military science, et cetera, it was supposed that he was not a practitioner of medicine. It would seem to be about as reasonable to contend that he was not an active practitioner on this basis, as to argue that S. Wier Mitchell was not a doctor because he was also an authority on snakes. If Celsus did not practice

medicine in Rome, then the Romans certainly lost a great man in the healing art.

During the middle ages Celsus' works on medicine were completely lost sight of. In 1443 Thomas of Sarzanne, who was later Pope Nicholas V discovered the manuscript of the "*De Re Medicina*" in Milan and in 1478 it was printed. Between that date and 1769 editions of Celsus appeared in Latin and apparently there was some disagreement in these texts as to exactly what the author meant in many cases. In the year 1769 the edition of Targa came out and from that date there has been little disagreement regarding the author's meaning as this work seemed to have settled most of the discussions on this matter. For about a hundred years after this date most editions of Celsus were printed in Latin.

In the 18th and early 19th centuries several translations were made into English and one or two into French. The English translations have all been out of print for many years. At the present time there are five or six of these translations which are admirable, but these books can only be found in rare libraries or in old book shops in England or on the continent. A few of these editions will be enumerated here.

(1) Edition of James Greive, 1756, went through several editions; the third edition is dated 1814. There is a copy of this work in the Columbia University Library, New York, in the Kings County Medical Society Library, Brooklyn, and in the Surgeon General's Library in Washington.

(2) The edition of Alex. Lee which is a translation of Targa's Celsus from the Latin of 1769. This is an admirable translation; two volumes bound in one with the Latin of Celsus and an *ordo verborum*, the purpose of which was to aid those students who were not familiar with classical Latin. This edition has an excellent English translation and wonderful notes on the entire eight books with a complete index, an index of the authors quoted, the life of Celsus, et cetera. This most excellent edition of the works of Celsus was published between 1831 and 1836. There is a copy of this in the Surgeon General's Library, the Kings County Medical Society Library and in the Library of Congress. It is rare.

(3) The Works of Celsus adopted for Students of Medicine by J. W. Underwood, 1830. This work is in two volumes and the translation is on the inter-linear plan which gives the volumes a very ragged and difficult appearance and, I should think, would not have been popular with the students of those days when the translation was made (1829 to 1833). Copies of this edition are to be

found in the Harvard College Library and in the Kings County Medical Society Library.

(4) The First Four Books of A. Cornelius Celsus *De Re Medica*, with an *Ordo Verborum* and literal translation by John Steggall. This is the least satisfactory of the several English translations I have seen. The second edition of this work was published in London in 1843. There is a copy of it in the Kings County Medical Society Library.

(5) One of the most satisfactory renderings of the Works of Celsus is that translated into French in 1876—*Traité De Médecine De A. C. Celse*. Traduction nouvelle, avec texte latin, notes, commentaires, tables explicatives, figures dans le texte, et quatorze planches contenant, 110 figures d'instruments de chirurgie antique, trouvés dans fouilles de villes Gallo-Romaines, de Pompéii et d'Herculanum; Par Le Dr. A. Védérès, Médecin Principal de l'Armée, chevalier de la Légion d'Honneur, de l'ordre de Pie IX, précédé d'une préface par Paul Broca. Professeur a la Faculté de Médecine de Paris, Membre de l'Académie de Médecine. Paris: G. Masson, Editeur Libraire de l'Académie de Médecine, Place de l'école de Médecine MDCCCLXXVI.

(6) Lastly, there are the several editions of the translation of G. F. Collier. The third edition of this translation came out in 1838. It is a very good edition of the work and is fairly common in the old book shops abroad. It has only the English text of the translation. The third edition is a duodecimo volume of about 370 pages and has some twelve or fourteen illustrations.

(7) All of the above mentioned translations are in the library of the New York Academy of Medicine.

Some of the expressions of Celsus are as fresh and frank as one could wish from a medical scientist. Take for instance, the opening paragraph of the section on "Diseases Incident to the Parts of Generation, and their Treatment."

The next diseases are those that affect the private parts; the nomenclature of which among the Greeks is not only tolerable, but now fully sanctioned by practice; for they are freely employed in almost every volume, work, or treatise of the physicians: but with us Romans, these terms are certainly filthy, and never employed by anyone who has a proper regard for modesty in language: therefore it is evident from this explanation, that there is no small difficulty in maintaining at the same time a delicacy of expression while delivering the precepts of the art. Not that this circumstance ought to deter me from treating on them: first, because it is my intention to comprehend everything in this work which I have found to be conducive to health; in the next place, because every person ought to know the treatment of those maladies which we so reluctantly expose to the view of another.

If the advice set forth in this paragraph had been followed out through the ages down to the present time, how much further advanced would this subject be than is actually the case. Few qualities of the human temperament have delayed our knowledge of medicine more than that of false modesty and a tendency to conceal from the physician those things which he should know and which he should publish for the benefit of his successors in the profession.

One may see in the consideration of several conditions of which Celsus speaks a complete history of what we now call syphilis but which the author thought were different diseases. For instance, in Lib. VI, Cap. XVIII, p. 203 of Lee's edition, the hard and soft genital sores are quite accurately described. Under the disease called *Porrigio*, Lib. VI, Cap. II, p. 132, the author describes an alopecia which he says is always accompanied by an underlying dyscrasia. In Lib. VI, Cap. III, p. 133, Celsus describes "*sycosis*" and in his description it is evident that several diseases are confused, but one may easily see the same type of lesion which he describes for other parts of the body under the name of "*ficus*." It is the condyloma usually occurring between the nates in untreated syphilis. Then, with the discussion of ulcers of the palate, Lib. VI, Cap. II, p. 190, one may easily construct the symptomatology of syphilis which seems so evident from many other parts of his work.

In Lib. III, Cap. II, p. 182 occurs the following which seems as fresh as the morning dew. Under the "Treatment of Fever with Concomitant Symptoms" in the fourth paragraph occurs the following sentence—"Now there are four diagnostic marks of inflammation, *redness*, and *swelling*, with *heat*, and *pain*." In Lib. V, Cap. XXVII, pages 88 and 89, Celsus considers the treatment of snake bite and though he did not have any antivenene to help him out, his treatment of the condition is just about as satisfactory if not more effective than the present day attempts to use these antivenenes for the cure of snake bite. The treatment was the incision and cupping of the area bitten. As we know, at the present time the surgical treatment of snake bite as developed by

Jackson, Harrison and others is far more satisfactory than the treatment of the condition by biological products.

Professor E. R. Long in "Readings in Pathology," says that Books IV and V are particularly rich in pathological observation and that Book IV "is essentially a special pathology in which disease is considered in relation to the individual organs."

It is our belief that the wide spread knowledge of Celsus which will be available when the Loeb translation above referred to comes out, will give us a better perspective and greater respect for the men of our profession who lived in ancient times. Certainly it will take some of the pride out of present day practitioners to see what Celsus actually knew about disease and its treatment.

—C. S. BUTLER.

NEWS AND NOTICES

TWELFTH ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

June 9 to 12, 1932, Milwaukee, Wisconsin

The twelfth annual session of the American Society of Clinical Pathologists held in Milwaukee marks an epic in the history of the Society in its contribution to medical science in the field of medico-legal literature. The scientific program with its outstanding Symposium on the medico-legal necropsy was full of interest. From the opening paper to the closing one the audience was kept in constant attendance despite the excessive heat experienced during the sessions. The usual congregating outside of the meeting room during the scientific sessions was conspicuous by its absence. Needless to say the 138 members and sixty visitors attending the meeting were well repaid for their trip to Milwaukee despite the weather.

The Symposium on hematology was another outstanding feature in which the activities of the hematology Registry were reported by Dr. R. R. Kracke and brought to a climax by the excellent presentation of Dr. William Bloom which brought to the attention of every clinical pathologist the importance of tissue culture in the study of diseases of the hematopoietic system. Also this year the scientific program was well balanced by an excellent group of papers dealing with histopathology.

The committees on scientific and commercial exhibits were certainly well repaid for their efforts this year. The first award for scientific exhibits was made to the group exhibit presented by Drs. A. O. Gettler, H. S. Martland, C. Norris and A. V. St. George. This exhibit was intensely interesting as it illustrated graphically and by actual specimens an unusual variety of examples of lesions met with by those dealing with medico-legal

necropsies and also a graphic demonstration of the various agents producing such lesions. The second award was made to Dr. W. D. Stovall for an excellent exhibit demonstrating a new classification of fungi by bacteriologic and pathologic methods. The exhibits in pathologic anatomy were well chosen and presented including some rare specimens. Hematology was as usual well represented by a large series of demonstrations by slides and pictures done in natural color. The art of color photography was well illustrated in a beautiful series of slides personally prepared by Dr. O. Lohr.

The annual banquet was attended by over 200 members and visitors and was an outstanding feature of the meeting. Dr. N. Enzer acted as toastmaster and gave an excellent exhibit in the science of public speaking keeping the audience in unusually high spirits and handling the procedures of the meeting with a strategy possessed only by masters of the art. The presidential address "The Clinical Pathologist as Teacher and Consultant" given by Dr. W. M. Simpson was intensely interesting and no doubt will be read by every member. The annual Ward Burdick Award was presented at this time to Dr. A. H. Sanford of Rochester, Minnesota. Following extemporaneous remarks by Dr. M. T. MacEachern of the American College of Surgeons and Dr. W. D. Cutter of the American Medical Association, the evening address was presented by Mr. Joseph A. Padway on "A Lawyer Looks at the Medical Profession." This address is of sufficient interest to our membership and to the medical profession as a whole that it will be published together with the Symposium on the medico-legal necropsy.

The business session unfortunately was not well attended. It is hoped that in the future plans will be made so that the distraction caused by the other meetings taking place on this day will be avoided. The following are the minutes of the business session.

The business meeting of the Society was held at the Hotel Pfister on Monday, June 12th. The meeting was called to order by President W. M. Simpson. It was moved and seconded that the reading of the minutes be dispensed with since they had previously been published in the JOURNAL.

REPORT OF THE SECRETARY-TREASURER

Since the activities of the Society have been summarized from time to time by means of circular letters to the membership and news items published in the Journal and the separate Committees will report later on the activities of the Society, I will make my report very brief.

Your Secretary is very grateful for the splendid coöperation he has received from the various Committees and individual members. The President has been particularly active this year because of the numerous problems that have arisen. His leadership and untiring efforts deserve the unanimous gratitude of our membership. The response of the local Counsellors to his call for enlargement and improvement of our membership is well demonstrated by the number and type of applicants to our Society for this year. The total active membership is 353 up to June 1, 1933. Of these 280 have paid their annual dues, fifty-six are in arrears for the current dues, sixteen owe for two years and one for three years. Five members have resigned and sixteen have been suspended for non-payment of dues.

The financial status of the Society despite the past unfavorable conditions is slowly but surely growing as evidenced by the fact that the net worth of the Society in July of 1930 was \$2696.00 as compared with the \$4942.00 of June 1, 1933. This amount represents actual cash assets in bonds or cash in the bank. Of this amount \$1638.43 is for the present unavailable due to the fact that the bank is being operated by a conservator. However I have been informed by reliable sources that the Society will not incur any losses on this amount.

Thanks to the coöperation of our membership in prompt payment of dues, the Society has been able to maintain its activities and still furnish each member with a year's subscription to our official Journal without increasing the dues despite the fact that the Journal costs the Society more than half the amount of the membership fee.

The following is a portion of the auditor's report:

BALANCE SHEET—MAY 29, 1933 AND STATEMENT OF INCOME AND EXPENSE FOR
PERIOD FROM APRIL 20, 1932 TO MAY 29, 1933

Assets

Balance in Citizens National Bank—Current Account \$1,313.54

Investments:

| | | |
|--|---------------|----------|
| Commonwealth Edison Co., No. 41022, 4 per cent Gold Bond, Series F, Due March 1, 1931, Par Value \$1,000.00 | \$945.00 | |
| American Telephone & Telegraph Co., No. 70378, 5 per cent Col. Trust Gold Bond, Due December 1, 1946, Par Value \$1,000.00 | 1,025.00 | 1,970.00 |
| <i>Furniture and fixtures</i> | \$601.75 | |
| <i>Less—Depreciation Reserve</i> | <u>581.21</u> | 20.54 |

Balance in Citizens National Bank:

| | |
|---|-------------------|
| Withdrawals discontinued by order of Treas. Dept... | 1,638.43 |
| <i>Total</i> | <u>\$4,942.51</u> |

*Liabilities**Balance Due Williams & Wilkins:*

| | |
|---|----------|
| Checks Issued prior to Bank Moratorium..... | \$325.00 |
|---|----------|

Net Worth

| | |
|-------------------------------------|------------|
| <i>Balance</i> —April 20, 1932..... | \$4,363.06 |
|-------------------------------------|------------|

Income:

| | |
|------------------------------|-------------------|
| Initiation Fees..... | \$425.00 |
| Membership Dues..... | 3,035.00 |
| Interest on Investments..... | 91.92 |
| Commercial Exhibit..... | 85.00 |
| <i>Total</i> | <u>\$3,636.92</u> |

Expense:

| | |
|---|-----------------|
| Salary..... | \$650.00 |
| Office Expense..... | 12.28 |
| Printing and Multigraphing..... | 166.65 |
| Postage, Telephone and Telegraph..... | 95.00 |
| Convention Expense..... | 191.62 |
| <i>Journal:</i> | |
| Editorial Office..... | 500.00 |
| Williams & Wilkins..... | 1,510.00 |
| Loss on Bond..... | 26.85 |
| Audit..... | 41.00 |
| Bond..... | 12.50 |
| Awards..... | 47.72 |
| Safety Deposit Box Rent..... | 3.30 |
| Miscellaneous..... | 2.40 |
| Exchange..... | 1.94 |
| Check Tax..... | .86 |
| Depreciation on Furniture and Fixtures..... | 120.35 |
| | <u>3,382.47</u> |

| | |
|--|---------------|
| <i>Income in Excess of Expense</i> | <u>254.45</u> |
|--|---------------|

| | |
|-----------------------------------|----------|
| <i>Balance</i> —May 29, 1933..... | 4,617.51 |
|-----------------------------------|----------|

| | |
|--------------------|-------------------|
| <i>Total</i> | <u>\$4,942.51</u> |
|--------------------|-------------------|

Motion made and seconded for acceptance of report. Carried.

REPORT OF COMMITTEE ON PUBLIC RELATIONS

The relations of the clinical pathologist with the public are not simple, nor are they easily defined.

In other years we have tried to put into words our ideals of service; to clarify in our own minds our relations with the laity and with our fellow physicians, and

to correct by education and organization evils which we have seen were threatening the welfare of the profession. We have expressed our desire to serve as consultants; we have discouraged the control of clinical laboratories by technicians; we have deplored the doing by hospital laboratories of work in competition with independent clinical pathologists; and some of us have been disturbed by the encroachment of Public Health laboratories upon the diagnostic laboratory service which the practicing clinical pathologist is prepared to furnish.

One year ago your Committee on Public Relations, its chairman having had successful experience in opposing an overzealous State Laboratory, presented suggestions which were adopted by the Society, and were recommended to its Counsellors for use in the various States where State Laboratory competition was annoying.

Your present Committee believed that its first duty was to find out what had been done by the Society's Counsellors in carrying out the recommendations made last year. Letters addressed to each of the forty Counsellors brought twenty-six replies and a significant variety of information. The details given would extend this report beyond reason.

In several States the battle has been carried on vigorously. The methods have varied according to the local situations and the personality of the combatants.

In all of the States heard from the State Laboratories are, or have been, doing more or less diagnostic work, not limited to communicable diseases. Reduced appropriations for public health work have made retrenchments necessary, but the practicing physician wants to save expense for his hard-pressed patients. This tends to keep up the volume of work sent to State Laboratories and the directors say they dare not refuse to do work sent to them. Some of the States have a population so distributed that no private laboratory service is available for many communities and the State help is really needed. In still other commonwealths so many clinical pathologists have been forced out by State competition, or by other conditions, that the few who remain can keep profitably busy with types of service which cannot be given by technicians or by an impersonal laboratory.

Several reports indicate that State Medical Schools may tend to join forces with Public Health Departments, and influenced by common ambitions develop an embryonic form of State Medicine with free laboratory service an early result.

Two approaches to the problem have been used by those who agree that there is a problem. The militant pathologist uses his political or persuasive skill and tries to secure an executive or a legislative order restricting Public Health laboratory service or a Commission to supervise such service. This has been done successfully in Indiana and Dr. Rhamy has told the Society how it was done. California has solved its difficulties in a different manner. In at least three other States somewhat similar attempts have so far failed to get results. Groups of physicians avowedly opposed to and fearful of State Medi-

cine have nevertheless voted against measures which would curtail State Laboratory diagnostic services. Legislators and State executives are sometimes not sympathetic with measures designed to protect an unwilling electorate.

The non-militant fraction of the Counsellors reporting have favored, some very decidedly, a program of professional improvement for ourselves, and an educational program among the members of State Medical Societies. They argue that the improper use of State Laboratory facilities is due as much to the thoughtlessness or lack of vision of the physician who supplies the work, as to the desire of the laboratory director to increase his own opportunity for service.

The question remains a many-sided one. We are presenting, as a supplement to this report, a group of resolutions which, at the discretion of the Society's Counsellors may be used by them as a guide in presenting this subject to their State Medical Societies.

The American Society for the Control of Cancer has suggested that there should be in each State at least one tissue pathologist thoroughly competent to recognize malignancy. This suggestion implies, and correctly, that not all pathologists who undertake to diagnose tissues are expert in the recognition of malignancy. It also implies that someone is to certify to the competence of at least one surgical pathologist in each State. This Society is vitally interested in any measure which will insure an earlier and more accurate diagnosis of cancer and should be able to furnish and certify proper pathologists for tissue diagnosis. We should encourage thorough special training for tissue pathologists and a minimum amount of supervised experience before independent diagnoses are accepted.

Two of the States reporting already have Cancer Commissions, and one is doing much in offering training and encouragement to the practicing clinical pathologists. State or district pathological conferences modeled after those already being held in California can well be arranged in many parts of the country.

It is fitting to mention in this report the work done by the Committee on the Costs of Medical Care. The Committee in its report has given deserved recognition to the value of laboratory procedures as aids in diagnosis. It calls attention to the increasing use of the laboratory as the patient's economic status rises; and that the amount of laboratory aid used even by the most prosperous group falls far below the ideal. The majority report then goes on to state repeatedly that laboratory service is a proper public health function; to list "laboratory technicians" as an essential part of proposed free medical services; and to suggest that under the new deal the general practitioner "could continue his present functions in much the present method but would utilize extensively diagnostic clinics or State Laboratories." The first minority report in cautioning against the "constant temptation in many fields to permit technicians to perform duties entirely unjustified by their knowledge and training" agrees perfectly with the frequently expressed attitude of this Society.

Much emphasis has been placed upon a statement that it is laboratory

charges which make medical care cost more now than it did in the nineteenth century. This is at least partial truth. We believe that the added immediate expense often results in an ultimate saving. With medical economists hunting industriously for ways to fit proper medical care into the budgets of all economic groups, we will doubtless be compelled to work with them for a solution or else risk the forcing of arrangements made without our help. The public must be served.

As a definite program for our membership in its public relations we would recommend:

- (1) The continued maintenance of the highest professional standards in our own work.
- (2) The wise use of our influence to encourage legislation which will help preserve efficient laboratory service for the medical profession.
- (3) Wider contacts between our members and other physicians to the end that they, as our associates, may know that as medical consultants and laboratory directors we are worthy of our hire.
- (4) Active coöperation with all attempts to furnish more accurate tissue diagnosis.
- (5) An official offer of coöperation by this Society in any organized plan for adjusting the economic side of medical care to the national budget.

RESOLUTIONS CONCERNING STATE LABORATORIES

WHEREAS, It is the recognized purpose of the Boards of Health of the various States to conserve the public health by measures for the prevention and control of communicable diseases, and

WHEREAS, Laboratories under the direction of many State Boards of Health have extended their activities beyond this field, for various reasons, and

WHEREAS, It is believed that the welfare of the public and of the medical profession will be best conserved if the activities of Public Health Laboratories are confined to measures for the prevention and regulation of communicable diseases, and to laboratory service for the indigent sick and for legal wards of the State,

Be It Resolved That two propositions be recommended for the consideration of State Medical Societies.

1. That the furnishing of general diagnostic service by State Laboratories tends to prepare the public conscience for the acceptance of added varieties of State medical practice.

2. That while the cost of true public health laboratory service is a legitimate governmental expense, the extension of this service into the field of general laboratory diagnosis is an unnecessary and unwarranted burden upon the taxpayers of the State.

These two points for criticism in the State Laboratory situation are very apparent in clinical pathologists and are worthy of consideration by the entire medical profession. It is unwise to ask the State to practice medicine in the

laboratory unless we are willing for it to spend still more public money and practice medicine in the ward and in the operating room.

The State Medical Societies can, and we believe they should, exert a powerful influence in keeping the demands made upon State Laboratories within the limits of truly public health functions. The Societies also can encourage the State Boards of Health to keep general diagnostic work out of their laboratories by regulation, and to inquire that each specimen from an indigent patient be accompanied by a card signed by both physician and patient. On this card the patient shall state: "I declare on my honor that I am financially unable to pay for this laboratory test and I know that the State Laboratory makes no charge for acid tests." The physician's statement shall read: "I state, on my honor, that this patient is financially unable to pay, and that I am making no charge for my services to this patient."

C. W. MAYNARD, *Chairman.*

Motion made and seconded for acceptance of report. Carried.

It was further moved and seconded that a copy of this report be sent to each Counsellor of the Society, the American Medical Association, the American Surgeons, American College of Physicians, Secretaries of the State Boards of Health and Directors of the State Boards of Health. Carried.

Moved and seconded that a letter be sent to the Presidents and Secretaries of the State Boards of Health who have contributed by their recent actions in the movement to withdraw services in the general field of clinical pathology and have attempted to restrict their activities to strictly public health problems. Carried.

REPORT OF EDITORIAL COMMITTEE

Volume 2 of the Journal has been completed in a satisfactory manner. As was thoroughly expected there was a falling off in subscriptions and advertising. Nevertheless, this was accomplished without the loss of good will and most cancellations have been due purely to financial conditions. In spite of this it was possible to furnish fifty extra pages and to make no charges for illustrations.

Owing to the record the Journal has made for the first two volumes the Williams and Wilkins Company is willing to set aside the original contract for the second time and as is shown in the enclosed letter has granted a more favorable contract to the Society. I recommend its adoption.

The Editor has been embarrassed by the necessity of returning dozens of manuscripts because of the lack of space in the Journal. It is also impossible to publish manuscripts as promptly as desired for the same reason. Nevertheless, until the financial situation clears up more, I recommend that no change in general policy be undertaken.

I invite the Executive Committee's attention to the necessity for the appointment of an editor and editorial board for the next three years beginning January 1, 1934.

T. B. MAGATH, *Chairman.*

Motion made and seconded for acceptance of report. Carried.

REPORT OF THE COUNCIL OF BIOLOGICAL ABSTRACTS

The meeting was held in Atlantic City with a very good attendance. The Editor reported excellent progress and support from the Rockefeller Foundation. A very unique and complete index to Volume II has been published and the other indices are now assured. Biological Abstracts has become extremely important in medical literature especially in pathology and is getting more so with each issue. The journal is to be strongly recommended to members of our Society.

T. B. MAGATH, *Representative*.

Motion made and seconded for acceptance of report. Carried.

REPORT OF THE BOARD OF REGISTRY

We submit herewith a report of the activities of the Registry for the twelve months ending April 30, 1933:

The past year has shown an unprecedented increase in the number of technicians applying for registration. No less than nine hundred have been added to our roster of which 893 were Laboratory Technicians and eleven received the classification of Medical Technologist. Our total registration now numbers 1949, and about 250 applications more are pending.

The phenomenal growth of the Registry and the increasing influence it exercises in medical and hospital circles are due to the cordial coöperation given us by the Fellows of the Society not only in having their own technicians registered but in giving freely of their time and labor in interviewing applicants referred to them for investigation. We have also been greatly aided in the hospital field by the representatives of the American College of Surgeons who, in their systematic inspection of hospitals, have emphasized the necessity of registering the laboratory workers under the auspices of the American Society of Clinical Pathologists. Similarly the American Medical Association has given the Registry recognition of its aims to maintain properly qualified laboratory technicians. The administrators of approved hospitals in the United States have likewise given us their support, as evidenced by their generous response to our circularization of over 2000 approved hospitals in the United States.

Our aims and purposes have been ably presented before the recent annual meeting of the Council on Medical Education and Hospitals by our President, Doctor Walter Simpson, and his address evoked useful discussion of the topic of technicians' training. This important adjunct to our work, the investigation and appraisal of schools for technicians, has been under the direct guidance of Doctor Kano Ikeda from whose report to our board we desire to make the following excerpts: On the basis of the minimum requirements for training technicians the total number of schools approved by the Board is 44. The great majority of these are conducted under the apprenticeship system in a hospital or private laboratory affiliated with a hospital of one hundred beds or over, and supervised by a competent clinical pathologist. Six of these schools are conducted by

colleges of higher learning who likewise have hospital affiliations. Here the students spend four years leading to a degree with medical technology as a major study. It is the endeavor of the Board to have all the approved training schools maintain a high level of instruction. Schools conducted primarily for profit are not eligible for approval.

During the year, we inaugurated the policy of publishing an official roster of the Laboratory Technicians and Medical Technologists who received certificates from our Board, also a list of approved schools for training technicians. This directory, arranged both alphabetically and geographically, serves a useful purpose for the technicians, the clinical pathologists and hospitals.

The Registry has also issued a revised edition of its booklet giving the aims and purposes of the Registry and defining the rules laid down by the Board for the qualification of technicians. Both of these pamphlets will be cheerfully sent to any of our Fellows on request.

In order to secure an unbiased appraisal of the technicians applying for certification, the Registry is inaugurating a written and oral examination to be conducted by a Fellow of our Society residing in the locality nearest to the applicant. The cooperation, heretofore shown by our colleagues, encourages us in the belief that they will gladly second our efforts in maintaining a high scientific and ethical standard of our registrants.

Appended herewith is the financial report of our Registry for the year 1932-1935, checked by a certified public accountant, which shows a very satisfactory condition of the treasury. All surplus above the working capital will, as in the preceding year, by vote of the Board be invested in Government Bonds.

In the conservation of these funds, the Board of Registry mindful of the altruistic aims of its foundation by the American Society of Clinical Pathologists, is looking ahead to the time when with the diminution of new applications the present surplus will enable the Registry to continue its useful function for the benefit of the patient, the technicians and the medical profession.

The Registry of Technicians since its inception in 1928 has become the recognized authority on this continent in evaluating the competence of laboratory technicians and training schools. Indirectly, it has also thrown into greater relief the important rôle of the clinical pathologist and enhanced the prestige of the American Society of Clinical Pathologists.

PHILIP HILLKOWITZ, *Chairman.*

Report accepted as read.

REPORT OF RESEARCH COMMITTEE

During the year, under the auspices of Dr. B. S. Kline, positive results obtained by use of the Friedman Test in cases of malignant testicular tumor, and also other cases were reported which gave a negative result with a rabbit. Also, positive tests were reported in cases of ectopic pregnancy with intact placental tissue, while three tubal pregnancies showing degenerated chorionic villi gave

negative tests, as is shown in the table below. The members of the Society used various technics, relative to quantity of urine injected and the number of injections and the duration of the test. The accuracy of the Friedman Test for normal pregnancies has been fully established, and it is suggested that future results of Society members in this regard be discontinued. A modification in technic worthy of note is the use of morphine sulphate intravenously as an anesthetic by Dr. A. M. Young, the details of which are in the *Journal of Laboratory and Clinical Medicine* (in press).

The Hematologic Registry under the care of Dr. R. R. Kracke now contains eighty cases of various types of blood dyscrasias, chiefly leukemias. Of these, twenty-eight cases are monocytic leukemias, collected by Dr. Kracke from the authors of the articles printed on the subject, including both American and foreign authors, the material of which collection was demonstrated at the Scientific Exhibit of our Society and the American Medical Association. It is hoped that more cases be sent in so that they may be loaned to the various men of the Society for their personal study.

There has been so little response to the subjects under the other divisions of our Committee, that details will be omitted here.

Your Committee wishes to thank the members who have cooperated in their work, and the President and Secretary of the Society, who have given their loyal support.

A. G. FOORD, *Chairman*.

REPORT OF SEROLOGY DIVISION FOR 1932

The one matter dealt with in this division consisted in correspondence with Dr. A. S. Giordano, Secretary and Dr. W. M. Simpson, President of The American Society of Clinical Pathologists relating to a suggestion that a North American Serological Conference comparable to the League of Nations' Serological Conferences in Denmark and Uruguay be organized.

Inasmuch as several Wassermann technics and quite a number of flocculation tests developed in this country have not yet been tried under conditions of rigorous control as employed in The League of Nations' Serological Conferences, no definite conclusion as to their comparative value has been reached.

As stated by Professor J. Jadassohn, President of the last League of Nations' Serological Conference at Montevideo in 1931:

The serological methods employed in the diagnosis of syphilis thus constitute a most valuable means of combating the disease. These methods deserve, therefore, our constant attention, and their improvement is of capital importance. Despite the merits of those who have already assisted in this work, the results are not yet entirely satisfactory, inasmuch as mathematical accuracy has not yet been achieved in connection with any one biological reaction. The fate of the patients depends, however, to a large extent upon the accuracy of these methods and, in connection with the diagnosis of syphilis in particular, a method must be found

which furnishes with syphilitic sera as high a percentage as possible of positive results, and does not produce non-specific reactions.

The various methods recommended have hitherto often been open to objection for one or other reason and sometimes produced inexplicably divergent results when used by the different serologists. The Laboratory Conferences on the serodiagnosis of syphilis convened by the League of Nations Health Organisation in 1923, 1928 and 1930 were intended to demonstrate the causes of these divergencies and to determine the value of different methods; the discussions also gave a definite impetus to serological researches, as may be seen by the improvement in methods, particularly flocculation methods during the last few years.

A North American Serological Conference, therefore, would be valuable in deciding the comparative merits of the various complement fixation and flocculation test methods employed in this country, Canada and Mexico and be helpful in establishing a high standard of procedure in the serodiagnosis of syphilis and in directing future research in this field.

In conclusion, it is recommended that the Society consider the advisability of organizing a North American Serological Conference.

B. S. KLINE.

Reports accepted as read.

REPORT OF THE COMMITTEE ON NECROPSIES

In a study of the matter of an insufficient number of autopsies in the various New York City hospitals, the Public Health Relations Committee of the New York Academy of Medicine found the cause to be in large part due to the existing Autopsy Law and the widespread practice of undertakers to advise against permission for postmortem examination.

While a change in the law was secured which was not as liberal as had been wished, it did however stop the claiming of bodies by undertakers in the hope of locating relatives or friends to pay funeral expenses, and the prevention by them of autopsies in these cases.

The matter of securing the goodwill of undertakers to stop as far as possible the practice of advising patrons against allowing postmortem examinations, was undertaken by a Joint Committee of the N. Y. Academy of Medicine, the New York Pathological Society and the Metropolitan Funeral Directors Association. A much improved understanding has resulted and autopsies are now much more general than formerly. The details are both interesting and instructive and will be published in the monograph on autopsies to be issued by the Society.

In view of the facts stated, your Committee recommends that this whole matter have the attention of pathologists generally with the hope that a post-mortem examination can be more easily secured.

A. V. ST. GEORGE, *Chairman.*

REPORT OF THE BOARD OF CENSORS

A complete list of members approved and recommended by the board was published in the July issue of the JOURNAL.

It was moved and seconded that the President be empowered to appoint any Committee he sees fit during the ensuing year. It was particularly requested that a Committee be appointed for modification of the Constitution and By-Laws. Carried.

Motion duly seconded that a vote of thanks be extended to the Local Committee for a very enjoyable and profitable convention. Carried.

The meeting was adjourned at eleven-thirty A.M.

A. S. GIORDANO, *Secretary-Treasurer*.

The following Committees have been appointed:

Committee on Local Arrangements:

B. S. Kline, Chairman
Anna M. Young
Russell Haden

Scientific Exhibit Committee:

Russell Haden, Chairman
W. S. Thomas
H. C. Sweany

Necrology Committee:

J. J. Moore, Chairman
Herman Spitz
F. C. Payne

Publication Committee:

J. A. Kolmer, Chairman
Kano Ikeda
W. C. MacCarty

Round Table Committee:

F. H. Lamb, Chairman
J. H. Black
J. J. Moore

Program Committee:

A. S. Giordano, Chairman
Frank Heck
W. M. Simpson

Constitution Revision Committee:

J. H. Black, Chairman
W. S. Thomas
K. M. Lynch

Committee on Necropsies:

F. E. Sondern, Chairman
I. Davidsohn
S. P. Reimann

Public Relations Committee:

C. W. Maynard, Chairman
K. M. Lynch
B. W. Rhamy

Publicity Committee:

T. B. Magath, Chairman
A. S. Giordano
W. M. Simpson

Research Committee

R. R. Kracke, General Chairman

(1) Hematology Division:

R. R. Kracke, Chairman
Frank Heck
N. Rosenthal

(2) Tumor Registry:

O. A. Brines, Chairman
A. C. Broders
C. M. Hyland

(3) Slide Exchange:

N. Enzer, Chairman

(4) Serology and Hormone Tests:

B. S. Kline, Chairman

REGISTRY OF TECHNICIANS OF THE AMERICAN SOCIETY OF CLINICAL
PATHOLOGISTS

The Board of Registry of the American Society of Clinical Pathologists at its annual meeting, in Milwaukee, on June 8th, 1933, considered eighty-four applications for the advanced rating of Medical Technologist. After careful examination of each applicant's qualifications thirty-seven were granted this title.

The question of training schools for laboratory technicians was uppermost in the minds of the Board who had previously gone on record against acceptance of applications from commercial schools. The ultimate goal of the Board is to place the training of clinical laboratory technicians under the tutelage of universities and colleges of learning. At present, clinical pathologists with good hospital affiliations may take on a limited number of students for training under the apprenticeship system.

The American Society of Clinical Laboratory Technicians was organized in Chicago, June 12-13, 1933. The meeting was opened at 2:30 P.M., June 12, with an invocation by Rev. A. J. Cook, S.J., of Cook County Hospital, Chicago. This was followed by an address of welcome by Dr. J. J. Moore, Director of the National Pathological Laboratory, Chicago, and an illustrated lecture on Diseases of the Blood by Dr. Roy R. Kraeke, Department of Pathology, Emory University, Georgia. Ten states were represented by delegates, and six others by proxy.

Officers elected were: President, Miss Madge Baldwin, Illinois; President Elect, Miss Lueille Burns, Minnesota; Vice President, Miss Elizabeth Gambrill, Georgia; Secretary-Treasurer, Mr. Donald S. Bryant, Illinois.

A banquet was held at the Medinah Athletic Club, at 7:30 P.M., at which Dr. Philip Hillkowitz, Chairman of the Board of Registry, Denver, Colorado, gave an address on registration, followed by Dr. Kano Ikeda, Secretary of the above Board, giving a history of the Registry and the requirements for registered technicians and medical technologists. Dr. J. J. Moore served as master of ceremonies. The program was enlivened by vocal solos, readings, and folk dances.

All registered technicians are invited and urged to become members. This may be done by getting in touch with your local society, or with Mrs. Louise R. Wright, 4535 East 17th Avenue, Denver, Colorado.

THE PHOTELOMETER* AND ITS USE IN THE CLINICAL LABORATORY†

ARTHUR H. SANFORD, CHARLES SHEARD AND ARNOLD E. OSTERBERG

Respectively of the Section on Clinical Pathology, Division of Physics and Biophysical Research and Section on Biochemistry, The Mayo Clinic, Rochester, Minnesota

ERRATA

VOLUME 3, NUMBER 5, SEPTEMBER, 1933

Page 370: Legend to figure 1 should read "Lung Opened to Show Lesions."

Page 371: Legend to figure 2 should read "Section of Lung Showing Monilia (Gram's stain, oil immersion)."

* The Sheard and Sanford photelometer will be manufactured by the Central Scientific Company of Chicago, Illinois. Any patent rights granted to the inventors, as well as any royalties accruing to them from the manufacture and sale of the photelometer, will be assigned to the American Society of Clinical Pathologists. These applications for patent rights have been made in order to control the development, accuracy and serviceability of such instruments, so that those who acquire apparatus involving the principles which have been or are to be disclosed may secure satisfactory equipment.

† Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

Photo-electrometers with one stage of amplification, made according to the disclosures given in these papers, have been operated very satisfactorily at The Mayo Clinic for three to four years in both clinical and experimental investigations concerning hemoglobin. During this period more than 200,000 determinations of grams of hemoglobin per 100 c.c. of blood have been made.

In the last year we have extended our researches along two lines: (1) the development of an improved instrument, known as the photometer, employing a photronic cell and a constant source of illumination operating on the ordinary 110-115 volt alternating current circuits, and (2) the application of photo-electrometry to various types of solution and of various substances in solution which are chiefly of significance in clinical laboratories.

FUNDAMENTAL PRINCIPLES OF PHOTOMETRY (PHOTO-ELECTRIC PHOTOMETRY)

In order to avoid repetition of details concerning the fundamental principles of photo-electric photometry, reference should be made to the previously published communications which have been cited in the foregoing paragraphs. By way of generalized statement, however, it may be well to point out that the light-sensitive cell (whether it be photo-conductive, photo-electric or photo-voltaic) is an electrical eye which does not suffer from the elements of fatigue or uncertainty in the determination of the amount or equality of illumination (as obtained by methods involving matches of equality of illumination in colorimetry) as in the case of the eye of man. In general, the current which flows in the circuit containing the photo-sensitive cell is proportional to the illumination which the cell receives. If the photo-sensitive cell is illuminated by a source of light which can be kept constant, the reading on a sufficiently sensitive instrument (such as a micro-ammeter) will be a constant. When, therefore, a solution of a substance which contains a characteristic absorption band is placed between the source of illumination and the photo-sensitive cell, together with a selective spectral filter (which may be of special glasses, dyed gelatin films or appropriate chemicals in solution) which transmits light only in the region of the char-

acteristic band, the amount of light which reaches the photo-sensitive cell will depend on the concentration of the substance under test which possesses the specified absorption band. According to the laws of Lambert and Beer, the concentration C of a given substance will be directly proportional to the negative logarithm of the unabsorbed light I . Since the current A in the circuit containing the photo-sensitive cell is directly proportional to the illumination I which it receives, it follows that the concentration of the substance under investigation which possesses a characteristic absorption zone when in solution is directly proportional to the negative logarithm of the unabsorbed light or, in other words, to the negative logarithm of the transmitted light. Hence, if C_1 and C_2 represent the concentrations of two given solutions of the same substance, I_1 and I_2 the amounts of transmitted light and A_1 and A_2 the readings of the current on the measuring instrument, then

$$\frac{C_1}{C_2} = \frac{-\log I_1}{-\log I_2} = \frac{-\log A_1}{-\log A_2}$$

REASONS FOR DEPARTURE OF PRACTICAL PHOTELOMETRY FROM THEORY

The ideal conditions which we have just outlined are met but rarely in actual practice. Among the reasons which may be cited to account for these deviations are: (1) Difficulty in obtaining filters which are feasible for ordinary technical routine and which have transmission bands corresponding exactly to the characteristic absorption band or zone selected. The curve of transmission of the filter should be the mirror image of the curve of absorption. (2) Variability in or change of shape of absorption band, and therefore the values of the absorption of light for the various wavelengths involved, with degree of concentration of the material in solution. Strictly speaking, the law $C = -k \log I$ holds only under the condition that the measurements of the unabsorbed light are made at a specified wavelength, such as that corresponding to the maximal absorption of light. (3) Lack of linearity or proportionality in the relationship between intensity of illumination I and the current A developed.

However, in spite of these factors which cause departure from the theoretically ideal situation, as well as other factors which perchance should be cited, photo-electric photometry furnishes a rapid and accurate method for the determination of the concentrations of various substances in solution under as close an observance, as is practically possible, of those criteria which we have pointed out. An approximation to those criteria or conditions imposed for the highest accuracy possible permits the operator to obtain a curve (in general, to be classed as an exponential curve) showing the relationship between concentration and readings on the current-measuring instrument. Such data, in general, when plotted on semi-logarithmic paper, will not give a straight line throughout the course of the plotted curve, although in many cases such plots will disclose the fact that, for a considerable range of concentrations and readings of current, the logarithmic law of Lambert and Beer is obeyed within a small percentage of error. When such is the case, the operator may dilute the solutions in such definite quantities as to cause the readings of the current to fall on the straight line portion of the plot and thereby insure the greatest accuracy possible under the method. Or, on the other hand, the operator may not concern himself about these matters but may proceed to obtain a curve showing the relationship between concentration and readings of the current (such as curve 1, figure 3) using a spectral filter which corresponds as closely as possible to an absorption band or zone of the substance under measurement. From such a curve, he may make a tabulation of corresponding concentrations and current readings. Such a procedure doubtless would be the best routine practice in the clinical laboratory, since a reading of the current would be transferred immediately from the table to the corresponding value of the concentration of the material in solution.

DIAGRAMMATIC SKETCH OF THE MODUS OPERANDI OF THE PHOTELOMETER

Figure 1 illustrates the essential features and, in general, the modus operandi of the photelometer. A constant source of light

is operated from a specially designed transformer in order that the voltage at the lamp may be kept constant. An adjustable diaphragm serves to regulate the amount of light which enters the lens. The parallel or slightly convergent light passes from the lens through the solution which exhibits spectroscopically characteristic absorption bands or zones. The light then passes through a suitable filter which transmits light in a region corresponding as accurately as possible to an absorption band of the material in solution. The radiant energy which has been transmitted by the solution and the spectral filter falls on the

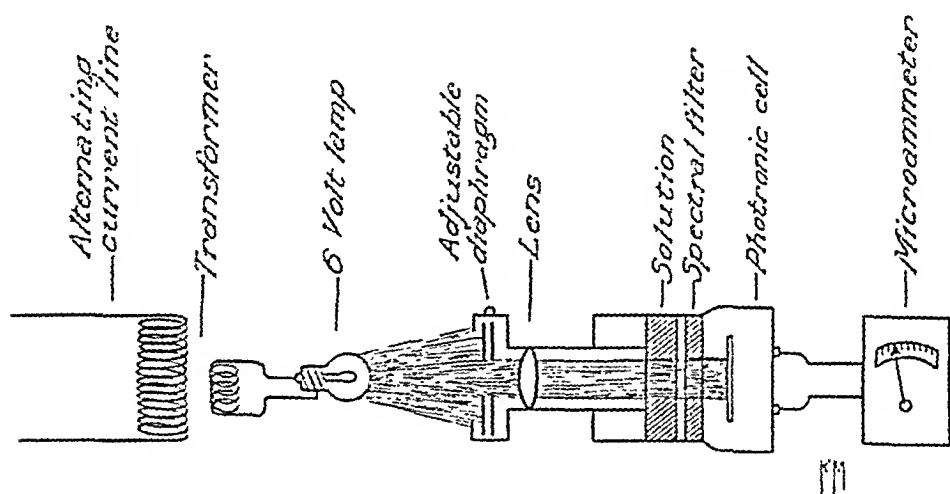


FIG. 1. DIAGRAM OF THE ESSENTIAL PHYSICAL FEATURES OF THE PHOTELOMETER

photronic cell and causes deflection of the current-measuring instrument. By varying the concentration of the material in solution the readings of the micro-ammeter may be converted into a graphic portrayal or tabulation showing the relationship between concentrations and readings on the micro-ammeter.

THE NEW IMPROVED PHOTELOMETER

The new type of photelometer differs from the photo-electrometer with one stage of amplification in two essential respects: (1) a photronic cell (Weston Electrical Instrument Company, Newark, N. J.) replaces the photo-electric cell (P. J. 24, General Electric Company or Westinghouse Electric Company) and the one stage of amplification, and (2) a specially constructed transformer,

so built as to keep the voltage (6 volts) delivered at the incandescent bulb constant within 1 or 2 per cent, and operating on the ordinary 110-115 volt alternating current circuits generally available in all communities.

Figure 2 is a view of the instrument* photographed directly from above and shows:

a. Lamp-house. This contains a 6-8 volt Mazda 50 candle power bulb and has a mechanism for adjusting the position of the lamp with reference to a double convex lens so that the light which falls on the photronic cell may be parallel or slightly convergent.

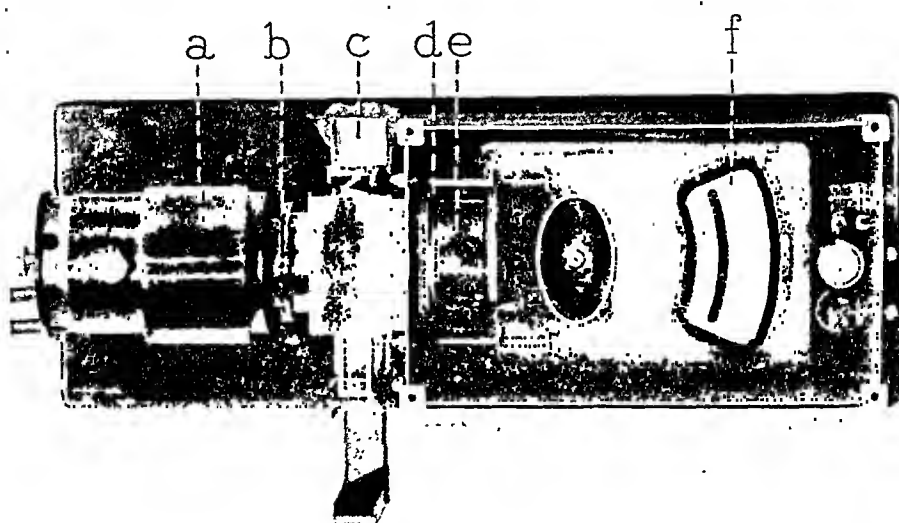


FIG. 2. THE PHOTELOMETER

a, lamphouse; *b*, iris diaphragm; *c*, carriage for holding solutions; *d*, spectral filter; *e*, photronic cell, and, *f*, micro-ammeter.

b. Diaphragm and lens. An iris diaphragm controls the amount of light which enters the lens placed immediately behind the diaphragm. The diaphragm is adjusted so that the light received by the photronic cell causes a

* Certain mechanical changes conducive to portability as well as to ease and certainty of operation have been incorporated since the time of the meeting of the Society in Milwaukee. These additional features are concerned with the proper mounting of the transformer on the base of the instrument, reduction in the number of switches, and the facility with which spectral filters or absorptive solutions may be changed and substitutes placed in proper position in front of the photronic cell.

deflection of the full scale of the current-measuring instrument (micro-ammeter) when a blank cell or container holding the solvent is placed between the source of light and the photronic cell.

c. Slide holder or carriage. This is for the reception of containers holding solutions to be measured. The movable carriage contains three compartments, one compartment of which may be used for the standard cell holding the solvent. The other two compartments are then available for rapid insertion of the solutions under examination. The containers for holding solutions are as uniformly as possible 1 cm. thick, as measured internally. Containers (spectroscopic absorption cells) which are quite uniform in this particular are obtainable commercially and at relatively small expense. It is obvious that the thickness of all solutions under test must be the same, otherwise the factor of change of thickness would have to be considered. Since the factor of thickness of solution is of importance, containers should be selected which have the same measurement of width (or thickness) when measured internally by suitable calipers. If cells of different internal thickness (D_1 and D_2) are used, correct calculations of the concentrations (C_1 and C_2) may be made in terms of the readings of the current (A_1 and A_2) on the micro-ammeter from the formula

$$\frac{C_1}{C_2} = \frac{\frac{-\log A_1}{D_1}}{\frac{-\log A_2}{D_2}} \quad \text{or} \quad \frac{C_1}{C_2} = \frac{-\log A_1}{-\log A_2} \times \frac{D_2}{D_1}$$

d. Spectral or absorptive filters. The spectral filters, whether of colored glass, Wratten filters or colored solutions, are placed directly in front of the photronic cell. The mechanical arrangement of the apparatus is such that the filter fits snugly against the photronic cell, thus preventing the admission of any stray light which might enter by reason of the incandescent lamp used to illuminate the scale of the micro-ammeter.

e. Photronic cell. The Weston photronic cell consists essentially of a thin metallic disk on which there is a film of light-sensitive material. The metal disk forms the positive terminal and a metallic collector ring in contact with the light-sensitive surface forms the negative terminal. There are no separate anode and collector plates, or evacuated or gas-filled space, as in the ordinary type of photo-electric cell. The action of light impinging on the sensitive cell appears to be entirely electronic. Tests made indicate that no chemical or physical change takes place, and therefore the life of the photronic cell seems to be unlimited. The current output of the cell is linear for values of the external resistance which are small (of the order of 10 per cent or less) when compared with the resistance of the cell. These cells, in the original form of manufacture, produce on the average about 1.4 micro-amperes per foot-candle of illumination uniformly distributed over the sensitive surface when connected to a relatively low external resistance. The latest type of cell contains no central nonsensitive area and is considerably more sensitive than the original form.

f. Micro-ammeter. The two wires leading from the photronic cell are connected to the positive and negative terminals respectively of the micro-ammeter (Weston Electric Instrument Co., Type No. 440), the scale of the instrument being divided into 100 parts. In order that the needle may be sufficiently damped to permit of rapid readings, a suitable external resistance (critical damping resistance) is properly placed in the circuit containing the micro-ammeter.

g. Lamp for illumination of the scale and magnifying lens. A 6-8 volt, 3 candle power bulb is used for the illumination of the scale. A lens, of low magnifying power and cut to an appropriate shape, is inserted in the cover of the instrument (fig. 2).

THE USE OF THE PHOTELOMETER IN THE DETERMINATION OF THE AMOUNT OF HEMOGLOBIN IN GRAMS PER 100 C.C. OF BLOOD

In order that photelometry may be carried out with accuracy in the determination of any substance three things are essential: (1) the solution must possess at least one absorption band or zone; (2) a spectral filter (Wratten, for example) or solution must be available which transmits light only in the region of one absorption zone of the material in solution which is under test, and (3) a method of obtaining a definite, known concentration of material in solution must be at hand to serve as a standard; this standard, therefore, by subsequent known degrees of dilution, provides a means of altering the concentrations in definite amounts.

Preparation of the sample of blood. Twenty cubic centimeters of the diluting fluid (0.1 per cent solution of sodium carbonate in water) are measured accurately into a suitable container such as a 50 c.c. centrifuge tube or Erlenmeyer flask. Preferably the blood should be taken from a vein. It is therefore most convenient to make the dilutions at the same time that the patient is being bled for tests on the serologic or chemical changes of the blood. The blood may be pipetted from a small tube immediately after it has been placed in it. Any long pipette with a capillary bore that is accurately marked for 0.1 c.c. may be used. Exactly 0.1 c.c. of blood is to be delivered by the pipette into the 20 c.c. of carbonate solution. It is obvious that this procedure must be done accurately and quickly. The tube is then shaken to facilitate complete dilution. The hemoglobin is converted immediately into oxyhemoglobin. Further details may be obtained from the paper by Sanford and Sheard.⁵

Spectrophotometric curve of oxyhemoglobin. Curves 2 and 3 of figure 3 show the characteristic absorption bands in the visible spectrum of oxyhemoglobin.

The curves give data for two concentrations of hemoglobin in water. The absorption bands have minimal transmission values at 578 and 542 millimicrons respectively.

Spectral filter. Curve 4 (fig. 3) shows the spectral transmission of Wratten filter number 74, and, furthermore, shows that it transmits light only in the region of oxyhemoglobin which has its maximal absorption at 542 millimicrons.

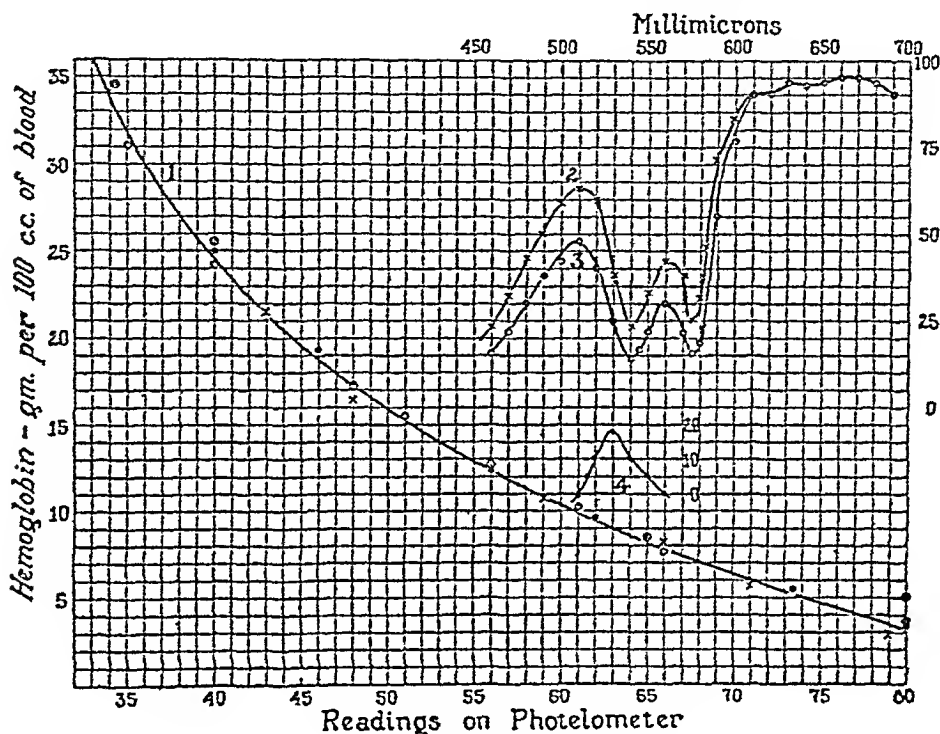


FIG. 3. DETERMINATION OF HEMOGLOBIN

Curve 1, relationship between the readings on the photometer and the grams of hemoglobin determined by the method of Van Slyke. Curves 2 and 3, spectrophotometric transmissions of diluted blood. Curve 4, spectrophotometric transmission of spectral filter used in the quantitative estimation of hemoglobin.

Standardization of hemoglobin. The oxygen capacity method of Van Slyke gives, by suitable calculation and with a probable error of about 1 to 2 per cent, the grams of hemoglobin for each 100 c.c. of blood. The reader is referred to the papers of Van Slyke^{4, 8} on gasometric determinations.

Correlation of grams of hemoglobin per 100 c.c. of blood and the readings on the photometer. Curve 1 of figure 3 gives data showing the values of the readings on the photometer in terms of the grams of hemoglobin per 100 c.c. of blood. Accurate determinations of the grams of hemoglobin were made of the blood

of several individuals by the Van Slyke method. Various but accurately measured amounts of blood were taken from the samples obtained at the time of the gasometric (Van Slyke) determinations and were diluted with 0.1 per cent sodium carbonate to 20 c.c. in each case. These dilutions of blood were then placed in the containers (1 cm. thick) which, in turn, were introduced into the sliding carriage of the photometer after the instrument had been adjusted to read a full-scale deflection (100 divisions) when a container holding the solvent only was inserted between the source of light and the photonic cell. The accuracy of the data of curve 1, figure 3, is dependent obviously on the accuracy of the determinations of content of hemoglobin by the method of Van Slyke, and on the skill and care with which the dilutions of samples of blood are made for use in the photometer.

The method of making a reading. The spectroscopic absorption cell is cleaned thoroughly with water, alcohol and ether. The container filled with water is placed in the middle compartment of the sliding carriage of the photometer and the iris diaphragm adjusted until the pointer of the micro-ammeter is exactly at the 100 mark. Containers filled with diluted blood are then inserted in the right and left compartments of the carriage and the carriage is moved and the readings of the micro-ammeter are made. The accuracy of setting of the micro-ammeter at the 100 division mark can be checked at any time if the container holding the solvent (water in this case) is allowed to remain in the middle compartment of the carriage. The reading on the micro-ammeter obtained by the insertion of diluted blood is translated readily into grams of hemoglobin in each 100 c.c. by referring either to the curve (fig. 3) or to a tabulation which can be made from the data of the curve.

In a series of experiments made to determine any fading effects due to standing of solutions of blood, it was found that there was less than 2 per cent change in the values of the grams of hemoglobin present after two or three hours' interval. Apparently any fading which occurs takes place during the first two or three hours, since readings taken four to eight hours later show no further changes. However, it is excellent practice, as well as a necessary procedure in measurement of certain types of solution, to make the readings as soon as possible after the dilutions are made.

THE USE OF THE PHOTOMETER IN THE DETERMINATION OF BLOOD SUGAR IN MILLIGRAMS PER 100 C.C. OF BLOOD

As examples of the quantitation of solutions of materials, other than hemoglobin, which possess color and therefore one or

more absorption bands or zones, we present the results obtained with the photelometer on the determination of dextrose in blood and of creatinine in blood. The blue solution for the determination of dextrose is obtained by the method (given in detail in succeeding paragraphs) of Folin and Wu.³

Principle. The protein-free blood filtrate is heated in a solution of alkaline copper, using a special tube to prevent reoxidation. The cuprous oxide formed is treated with a solution of phosphomolybdic acid. A blue colored solution is obtained.

Solutions. Solution 1, stock solution: Dissolve 2.5 gm. of benzoic acid in 1 liter of boiling water and cool. Transfer to a bottle; the solution will keep indefinitely. Dissolve 1 gm. of pure glucose in about 50 c.c. of the benzoic acid solution. Transfer to a 100 c.c. volumetric flask, rinse, and fill to the mark with the benzoic acid solution. This is the standard (1 per cent) stock solution. Label and preserve. This solution seems to keep indefinitely. Transfer 3 c.c. of the stock solution, by means of an Ostwald pipette, to a 200 c.c. volumetric flask; fill to the mark with saturated benzoic acid solution and mix. The diluted solution so obtained, which contains 0.15 mg. of glucose per cubic centimeter, is a suitable standard for most determinations of blood sugar. Use 2 c.c. for each determination.

Solution 2: Alkaline copper solution. Dissolve 40 gm. of anhydrous sodium carbonate in about 400 c.c. of water, and transfer to a liter flask. Add 7.5 gm. of tartaric acid, and when the latter has dissolved, add 4.5 gm. of crystallized copper sulphate; mix and make up to a volume of 1 liter. If the carbonate is impure, a sediment may be formed in the course of a week or so; in this event decant the clear solution into another bottle.

Solution 3: Special phosphomolybdic acid solution. Transfer to a liter beaker 35 gm. of molybdic acid and 5 gm. of sodium tungstate. Add 200 c.c. of 10 per cent sodium hydroxide and 200 c.c. of water. Boil vigorously for twenty to forty minutes so as to remove almost all of the ammonia present in the molybdic acid. Cool, dilute to about 350 c.c., and add 125 c.c. of concentrated (85 per cent) phosphoric acid. Dilute to 500 c.c.

Procedure for the preparation of the solutions for the quantitation of blood sugar. Transfer 2 c.c. of the tungstic acid blood filtrate to a blood sugar test tube, and to another similar test tube (graduated at 25 c.c.) add 2 c.c. of standard sugar solution containing 0.15 mg. of dextrose. To each tube add 2 c.c. of the alkaline copper solution. The surface of the mixtures must reach the constricted part of the tube. Transfer the tubes to a boiling water bath and heat for 6 to 8 minutes. Remove the sugar tubes and add immediately (that is, before cooling) 2 c.c. of the molybdate phosphate solution. When the cuprous oxide is dissolved, cool, and dilute the resulting blue solutions to the 25 c.c. mark, insert a rubber stopper, and mix. It is essential that adequate attention be given

to this mixing because the greater part of the blue color is formed in the bulb of the tube.

Quantitation of blood sugar by the photometer. Curves 2 and 3 of figure 4 show the values of the spectrophotometric transmissions, at wavelengths from 700 to 440 millimicrons, of two different concentrations of preparations for the determination of blood sugar. Both curves exhibit a definite absorption zone

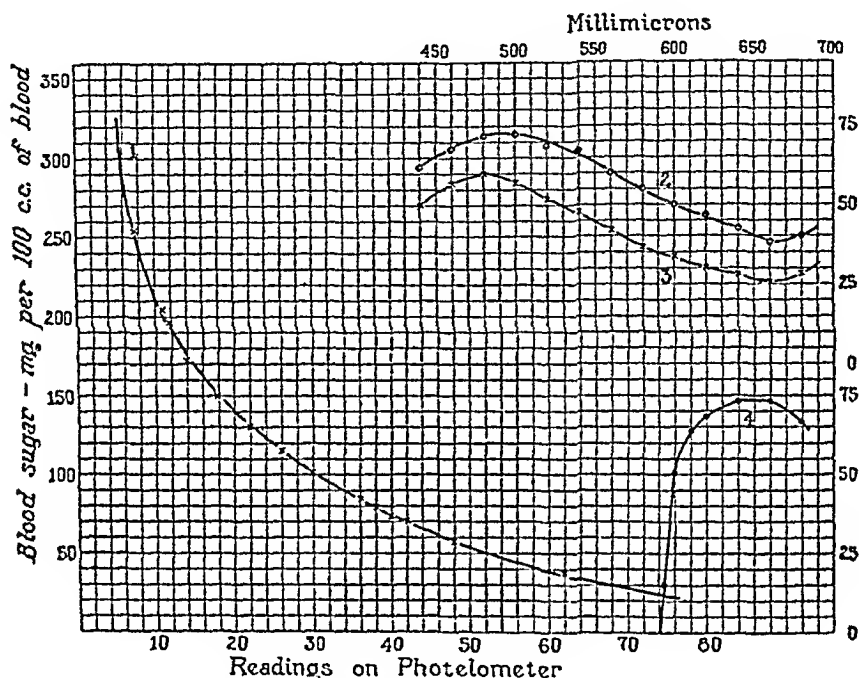


FIG. 4. DETERMINATION OF BLOOD SUGAR

Curve 1, relationship between concentrations of blood sugar and the readings on the photometer. Curves 2 and 3, spectrophotometric transmissions of blood sugar solutions prepared by the method of Folin and Wu. Curve 4, spectrophotometric transmission of the spectral filter used in the quantitation of blood sugar.

with a minimal transmission at approximately 660 millimicrons. Curve 4 shows the spectral transmission of Wratten filter number 29 and demonstrates that the filter transmits light in maximal quantity in the region 660 to 660 millimicrons, or in the region in which the preparations of blood sugar are markedly absorptive. Curve 1, figure 4, was obtained by plotting the values of blood sugars, as determined colorimetrically, as ordinates and the corresponding readings of the photometer as abscissae. Or, for purposes of calibration, definitely determined dilutions of samples of the standard solution may be made

and a curve plotted showing the relationship between milligrams of blood sugar per 100 c.c. of blood and readings on the photelometer. Because of fading or loss of color in solutions prepared for the determination of blood sugar, it is essential that a definite time schedule be adopted for the accurate determination of a curve similar to curve 1 of figure 4. For example, a ten-minute interval between preparation and reading on the photelometer may be selected. Irrespective of the regimen adopted, strict adherence to the schedule must be maintained in either colorimetric or photelometric estimations of solutions which change in color value on standing.

THE USE OF THE PHOTELOMETER IN THE DETERMINATION OF CREATININE IN MILLIGRAMS PER 100 C.C. OF BLOOD

The yellow solution of creatinine, obtained by treatment of protein-free filtrate with alkaline picrate (by the methods given here in some detail) may be quantitated by the photelometer.

Principle. By colorimetric methods, a portion of the blood filtrate is treated with alkaline picrate solution and the color developed compared with that of a standard. In making determinations of creatinine with the photelometer it is necessary to determine a curve showing the relationship between milligrams of creatinine per 100 c.c. of blood and the readings on the micro-ammeter.

Solutions. Solution 1: Stock standard creatinine solution is obtained by dissolving 1.61 gm. of creatinine zinc chloride in 1 liter of $N/10$ HCl.

Solution 2: Dilute standard creatinine solution. Transfer to a liter flask 6 c.c. of the stock standard creatinine solution, add 10 c.c. of normal hydrochloric acid, dilute to the mark with water, and mix. Transfer to a bottle and add 4 or 5 drops of toluene or xylene; 5 c.c. of this standard contains 0.03 mg. of creatinine.

Solution 3: Saturated picric acid solution is obtained by dissolving 1.50 gm. of purified picric acid in 100 c.c. of water.

Procedure for the preparation of solutions for the quantitation of creatinine. Transfer 25 (or 50) c.c. of a saturated solution of purified picric acid to a small, clean flask, add 5 (or 10) c.c. of 10 per cent sodium hydroxide and mix. Transfer 10 c.c. of blood filtrate to a small flask, or to a test tube, and dilute the standard to 20 c.c. Then add 5 c.c. of the freshly prepared alkaline picrate solution to the blood filtrate and 10 c.c. to the diluted creatinine solution. Let stand for eight or ten minutes and make the determinations with the photelometer. The photelometric determinations should be completed within a specified time (not over fifteen minutes) from the time the alkaline picrate was added. Therefore it is not advisable to work with more than three to five blood filtrates at a time.

In the case of unusual bloods representing retention of creatinine, take 10 c.c. of the standard plus 10 c.c. of water, which covers the range of 2 to 4 mg.

of creatinine per 100 c.c. of blood; or 15 c.c. of the standard plus 5 c.c. of water by which 4 to 6 mg. can be estimated. By taking the full 20 c.c. volume from the standard solution at least 8 mg. can be estimated.

Quantitation of creatinine by the photometer. Curves 2 and 3 of figure 5 show the values of the spectrophotometric transmissions in the visible spectrum of two different concentrations of preparations for the determination of creatinine. Both curves show a definite absorption zone in the short wave length region of the spectrum. Curve 4 shows the spectral transmission of Wratten

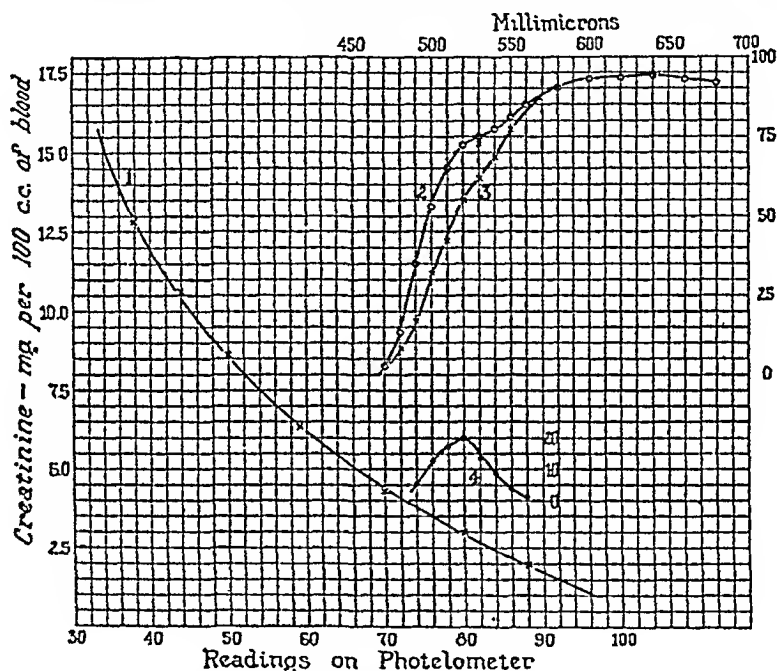


FIG. 5. DETERMINATION OF CREATININE

Curve 1, relationship between concentrations of creatinine and readings on the photometer. Curves 2 and 3, spectrophotometric transmissions of two solutions of creatinine. Curve 4, spectrophotometric transmission of spectral filter used in the quantitative estimation of creatinine.

filter number 63 and demonstrates that the filter transmits light in maximal quantity in the region 500 to 530 millimicrons or in a portion of the region in which the preparations of creatinine are markedly absorptive. Curve 1 was obtained by plotting the values of creatinine, as determined by dilution of a preparation containing 12.8 mg. of creatinine by colorimetric methods, against the readings on the photometer. The original creatinine preparation was diluted with amounts of tungstic acid to give values of creatinine corresponding to 10.6, 8.5, 6.4, 4.3 and 2.1 mg. per 100 c.c. of blood respectively.

Because of fading or loss of color in preparations of creatinine, it is essential to adopt a definite time schedule.

SUMMARY

Information and data have been presented concerning the following:

Fundamental principles of photo-electric photometry, with a résumé of their application, as previously made by us, to the development of the photo-electric hemoglobinometer and the photo-electrometer with one stage of amplification.

The development of a new type of instrument, the photelometer, using as a source of illumination a 6 to 8 volt, 50 candle power Mazda lamp operated at constant voltage by means of a specially designed transformer and a photovoltaic cell (pho-tronic cell), in direct combination with a micro-ammeter, as the optico-electrical system for measuring the amount of light transmitted by various substances in solution.

Details concerning the construction and operation of the photelometer, and such accessories as are essential or desirable for ease of operation and accuracy of results.

The advantages of the use of the photelometer and appropriate spectral filters in clinical and chemical laboratories, as illustrated by data and curves relative to the quantitative estimation of hemoglobin, blood sugar and creatinine.

The applicability of the principles of photo-electric photometry, as exemplified in the photelometer, to the quantitative determinations of various substances in solution which possess at least one characteristic absorption band or zone in the visible spectrum and also to the quantitative estimation of materials in suspension.

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FLOCCULATION REACTIONS FOR SYPHILIS*

COMPARATIVE RESULTS WITH SIX FLOCCULATION AND TWO COMPLEMENT FIXATION TESTS

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Innumerable series of tests have been made on blood specimens since the first complement fixation reaction for syphilis was reported by Wassermann in 1906. The almost simultaneous development of the flocculation principle by Michaelis for the diagnosis of syphilis started the controversy between the proponents of the two types, and this still persists. Refinements in the technic of these two types of tests have resulted in perhaps the most accurate diagnostic laboratory procedures known for any one condition.

The constant urge that these tests be made more sensitive and more simple has developed the large number of modifications of both the complement fixation and flocculation or precipitation tests, with only a few attempts toward standardization.

Realization that both types of tests are physico-chemical in nature, and in principle are quite if not entirely similar may result in ultimate standardization to one or two tests of each type. The appreciation that the same variables are largely present in the different tests, as stated by Eagle,² and that experience by trial and error should fix these variables within narrow limits may result in a closer approach to the ideal test as outlined by Kilduffe.⁶

Many of the published reports evaluate other tests in terms of

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a favorite procedure. This is naturally true of reports by the originators of the different tests, and by their expert assistants, as both have developed a surpassing technic in the procedure in which they are particularly interested, as compared with other tests.

Perhaps every clinical laboratory with any considerable volume of work has run a series of blood specimens with different tests to determine the procedures most satisfactory for them. That idea has been carried out for several years in this laboratory, and has crystallized in the present report.

The present series of blood tests was carried out to determine the relative value of the different procedures in the hands of well trained, competent but not expert technicians.* Similar conditions of personnel undoubtedly prevail in many other qualified laboratories, also the majority of the reactions under discussion are reported by such laboratories. The different tests reported were carried out previously for varying lengths of time until dependable results had been obtained.

One thousand specimens of blood were run by the six flocculation or precipitation tests and the two complement fixation tests, as described below. All tests were done on the same specimen of blood, and for this purpose ten cubic centimeters of venous blood were obtained from a routine series of patients in the clinics and wards of the University Hospitals.

The WaR test is a complement fixation procedure which has been carried out routinely for almost twenty years in this laboratory, with but minor alterations. Two antigens, one cholesterolized, water bath incubation, and the sheep's cell system is employed. The Kolmer⁸ quantitative test has been carried out for several years with close adherence to the original technic.

The Eagle,¹ Hinton,^{3,4} Kahn standard,⁵ Kline,⁷ Meinicke (M.K.R.),¹⁰ and Müller (M.B.R. II)¹¹ tests have been carried out according to the described technic. Antigens† for the Eagle,

* An expert technician is considered in this discussion to be exceptionally well trained for a particular procedure, and who devotes the major part or all of his time to that procedure.

† Drs. Hinton, Kahn and Kline kindly furnished us with samples of their respective antigens which served as checks for the antigens prepared by us.

Hinton, Kahn, Kline, Kolmer and WaR tests as used in this series were prepared in this laboratory. The Meinicke and Müller antigens were purchased in the open market, as no adequate directions could be found for their preparation. The Kline antigen was used for both the diagnostic and exclusion tests.

Among the thousand cases were a number of individuals with a luetic history or positive physical findings for primary lesions, secondary eruptions, and various tertiary and congenital manifestations. No effort, however, has been made to classify the cases into groups other than to prove or disprove the presence of syphilis in every way possible.

TABLE 1
RESULTS OF THE VARIOUS TESTS ON 1,000 BLOOD SPECIMENS

| | ALL REACTIONS AGREE | | | ONE OR MORE REACTIONS DISAGREE | |
|-----------------------|---------------------|----------|----------|--------------------------------|----------|
| | Positive | Negative | Positive | Positive | Negative |
| History—luetie..... | Positive | Negative | Positive | Positive | Negative |
| Reactions..... | Positive | Negative | Negative | | |
| Number of bloods..... | 224 | 564 | 11 | 158 | 43 |
| Percent..... | 22.4 | 56.4 | 1.1 | 15.8 | 4.3 |
| Total percent..... | 79.9 | | | 20.1 | |

Table 1 enumerates the cases in which there was complete serological agreement, positive and negative, with the history; also complete disagreement. No distinction was made for the degree of positive reactions, as emphasized by Kolmer.⁹ Eleven blood specimens were from cases with a definite luetic history, and with treatment apparently sufficient to produce negative reactions. Partial serological agreement and the false positive reactions are considered separately. Those serological tests which gave positive reactions in which no possible evidence could be elicited of syphilis were considered as probably false.

In table 2 those cases with a positive luetic history but with disagreement in reactions are analysed. Many of these cases had had more or less treatment, and were largely tertiary or

latent in clinical manifestations. Two cases with primary lesions and a positive darkfield examination for *Treponema pallidum* were in this group, with the syphilitic reagin apparently just beginning to develop. The balance of the group shows that a large majority gave positive reactions of varying degree with five or more tests. The relative order of sensitivity is interesting, as it shows that in this group the flocculation tests gave definitely the larger number of positive reactions. It is in these types of cases that the tests have their greatest value, to deter-

TABLE 2

REACTIONS OF 158 BLOODS WHICH GAVE VARYING RESULTS WITH DIFFERENT TESTS

Luetic history, positive

| TEST | POSITIVE | PERCENT OF 1,000 BLOODS | ORDER OF SENSITIVITY |
|----------------------------|----------|----------------------------|-------------------------|
| WaR..... | 20 | 2.0 | 8 |
| Kolmer (quantitative)..... | 86 | 8.6 | 7 |
| Eagle..... | 116 | 11.6 | 4 |
| Hinton..... | 118 | 11.8 | 3 |
| Kahn..... | 98 | 9.8 | 6 |
| Kline..... | 135 | 13.5 | 1 |
| Meinicke..... | 129 | 12.9 | 2 |
| Müller..... | 111 | 11.1 | 5 |

Total positive reactions for this group: 813

Positive reactions per blood: 5+

mine diagnosis and to control treatment. But in this group the greatest difficulties arise.

The degree of sensitivity which apparently can be more or less controlled has been another point of controversy in these tests of non-specific nature. The ideal test will give no false positive reactions. Forty-three blood specimens from cases with non-luetic histories gave fifty-nine positive reactions of varying degrees with the different tests, as shown in table 3. The Kline and Müller tests gave very definitely more positive reactions in these cases. This is not parallel, however, to the order of sensitivity as shown in table 2. This difficulty of the hypersensitive

antigen and the false positive reaction is considered by Kilduffe as a problem for the clinician, who can best evaluate the laboratory results. Error in technic may account for a certain number but not all of the erroneous results obtained.

TABLE 3
ONE OR MORE REACTIONS POSITIVE
Luetic history, negative

| TEST | NUMBER OF FALSE POSITIVE REACTIONS |
|--------------------------|--|
| WaR | 1 |
| Kolmer quantitative..... | 1 |
| Eagle..... | 1 |
| Hinton..... | 1 |
| Kahn..... | 3 |
| Kline..... | 19 |
| Meinicke | 3 |
| Müller..... | 29 |
| Total..... | 58 |

Number bloods in this group: 43
Positive reactions per blood: 1+

COMMENTS

The impressions gained from previous work and from this series of tests are that the Kolmer quantitative test is the most valuable complement fixation procedure for the diagnosis of syphilis we have used. The technic is, however, quite difficult, and this unfortunately must prevent its more general use.

Among the flocculation tests the Eagle technic stands out for its relative simplicity, specificity, and ease of reading. The Müller reaction also is easily read, but has given us a much greater number of non-specific reactions. The Meinicke test probably ranks next to the Eagle test, but the fact that this antigen and also the Müller antigen must be purchased without any knowledge of their age or care since they were prepared and with an apparent difference encountered in different lots all act to the disadvantage of both.

The Hinton and Kahn tests show a high degree of specificity, but both were found to be rather difficult to read. There is but little to choose between these two tests except perhaps the greater simplicity of the Hinton test with only one tube.

The use of the paraffin rings in the Kline test set this procedure apart from the others and while we found it an easy technic to carry out there was a definite tendency to give non-specific reactions. However in luetic cases it ranked first in sensitivity.

CONCLUSIONS

This series has shown the advantage of performing a complement fixation test and a flocculation test on every blood specimen as supplemental to each other.

The flocculation tests have a definitely higher degree of sensitivity than the complement fixation tests in treated and latent cases of syphilis.

The majority of the tests concerned have a high degree of specificity.

All eight tests require a high degree of technical skill, and increasing experience with any test enhances its value.

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A SURVEY OF 1000 GONOCOCCUS COMPLEMENT-FIXATION TESTS PERFORMED WITH THE SERUMS OF MALE PATIENTS IN AN OUTPATIENT CLINIC*

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The object of this study was to analyze the results of routine complement-fixation tests performed with the blood serums of male patients in a large clinic treating ambulatory cases only, and to establish, if possible, with the aid of a detailed clinical study, the practical value of this laboratory procedure. The analysis is based upon a study of 1000 tests performed with the serums of as many patients.

The gonococcus antigen used was a commercial preparation which consisted of a suspension of pure cultures of gonococci. It was prepared from a number of strains of the organism isolated from acute and chronic cases. The various strains incorporated in this antigen were so selected as to insure the applicability of the antigen in any cases of gonococcal infection. The procedure employed is outlined in table 1.

The tubes were placed in the ice-box over night, and the results were read the following morning. Depending upon the degree of the hemolytic inhibition, the results were recorded in accordance with the generally adopted scheme for complement-fixation, namely: four-plus, three-plus, two-plus, one-plus, trace or doubtful, and negative.

All the serums upon which this study is based were tested by the Kolmer complement-fixation and Kahn antigen precipitation tests in addition to the gonococcus complement-fixation.

The cases studied were grouped for the purpose of analysis in accordance with the results of the serologic tests.

NEGATIVE BY ALL THE TESTS USED

Serum specimens from six hundred patients, or 60 per cent of the series studied gave negative results by all the tests employed.

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Three hundred histories taken at random were examined. It was found that in seventy cases, or 23 per cent of the 300, the diagnosis of acute gonococcal urethritis was made; three, or 1 per cent, gonococcal prostatitis; three, or 1 per cent, gonococcal urethritis, chronic. Of the seventy cases of acute urethritis six, or 2 per cent of the 300, were diagnosed as posterior urethritis. Sixty patients, or 20 per cent of the 300, denied having had a gonococcal infection, while eighty-one, or 27 per cent of the 300, admitted one or more attacks. The period elapsing since the last attack of the gonorrheal infection among the patients who admitted having had the infection varied from one to twenty-

TABLE 1

| REAGENTS | 1ST TUBE | 2ND TUBE | CONTROL TUBE |
|---|----------|----------|--------------|
| | cc. | cc. | cc. |
| Patient's serum 1:5 dilution | 0.5 | 0.25 | 0.5 |
| Antigen 1:20 dilution | 0.15 | 0.15 | None |
| Saline | 0.35 | 0.60 | 0.85 |
| Complement 1:50 dilution | 1.0 | 1.0 | 1.0 |
| Incubate for one hour in a water-bath at 37.5°C. then add | | | |
| Sheep cells, 5 per cent suspension | 0.5 | 0.5 | 0.5 |
| Amboceptor 1 dose, 1:1000 dilution | 0.5 | 0.5 | 0.5 |
| Incubate for one hour in a water-bath at 37.5°C. | | | |

five years, and the number of attacks varied from one to six. All had received treatment, which, according to the statements given, resulted in complications of the usual type in a noteworthy percentage of instances. The examination of the patients whose condition was diagnosed as gonococcal infection was made from two days to four months following the date of the alleged exposure, the greater percentage varying from one to four weeks. Nothing directly or indirectly connected with the gonorrheal infection could be discerned from the histories of the remaining 50 per cent of the cases of this group.

The most pertinent points obtained in the above analysis are recapitulated in table 2.

The above analysis indicates that complement-fixation by the antigen consisting of a suspension of unwashed gonococci fails to yield positive results in 25 per cent of cases with acute gonococcal urethritis even as late as four weeks following the infection. Most of the cases had an anterior infection, though some posterior infections of the same duration likewise failed to react positively with the complement-fixation procedure as used in this study. Since nearly all the cases with a history of the gonococcal infection as far back as one to twenty-five years, with and without ensuing complications (but free from active infection), do not respond positively to the gonococcus complement-fixation, it

TABLE 2

| | <i>per cent</i> |
|--|-----------------|
| Negative gonorrheal complement fixation results were obtained in: | |
| Gonococcal urethritis, acute, anterior..... | 21 |
| Gonococcal urethritis, acute, posterior..... | 2 |
| Gonococcal urethritis, chronic..... | 1 |
| Gonococcal prostatitis, chronic..... | 1 |
| Total per cent of cases with active gonorrheal infection..... | 25 |
| Cases free from active gonorrheal infection, but with history of past infection..... | 25 |

appears reasonable to assume that the persistence of the gonococcus antibody is of a limited duration.

CASES POSITIVE BY THE GONOCOCCUS COMPLEMENT-FIXATION ONLY

Cases with strongly positive reactions. (Four plus—four plus to three plus—two plus, in the first and second tubes respectively)

Serums of ninety patients studied, or 9 per cent of the entire series, gave reactions of the intensities indicated above. In examining the histories of the patients whose serums are included in this group, the following important information was considered: (1) History of previous gonococcal infection, (2) date of exposure, (3) urethral discharge, (4) results of the two glass urine test, (5) microscopic urine examination, mainly for the presence of pus,

(6) glandular involvement, (7) examination of the prostatic discharge, and (8) examination of the urethral discharge by the methylene blue method.

The most important information gathered from the clinical histories of the ninety cases of this group can be conveniently presented as in table 3.

Forty-two of the ninety patients admitted having had previous gonococcal infections with periods of time varying from four months to fifteen years. No account was taken of the number of previous attacks of gonorrhea. The examination of the patients of this group was made from two days to six weeks following the

TABLE 3

| DIAGNOSIS | NUMBER OF CASES |
|--|-----------------|
| Acute gonorrheal urethritis | 41 |
| Acute gonorrheal urethritis and syphilis, treated, seronegative.. | 5 |
| Chronic prostatitis | 16 |
| Gonorrheal urethritis, posterior..... | 11 |
| Gonorrheal urethritis, prostatitis, and epididymitis..... | 3 |
| Gonorrheal urethritis, prostatitis, and epididymitis, and syphilis, treated, seronegative..... | 3 |
| Urethral gland infected..... | 1 |
| Left town (were under observation for gonorrheal infection) ... | 3 |
| Enlarged prostate (under observation for gonorrheal infection ... | 4 |
| Non-venereal..... | 3 |

alleged exposure. The strong gonorrheal complement-fixation reactions with the serums of the last three cases shown in table 3 indicate the possibility of either a nondisclosed history of a comparatively recent attack which may have been cured or suppressed shortly before the patients reported at the Institute, or of the reactions being non-specific. It is probable that upon successive repeated tests the reactions might be found to disappear spontaneously, as is the case with many of the non-specific fixation reactions obtained with the beef-heart antigen. This phase of the problem deserves a thorough study.

Generally, however, the analysis of the results of the serum-reactions included in this group indicates that strong and moder-

ate gonococcus complement-fixation reactions are predominately specific and indicate the existence of an active or recently active gonorrheal infection. This leads us to believe that cases suspected of having a gonococcal infection but lacking in clinical and bacteriological evidence, yet yielding strongly positive gonococcus complement-fixation reactions, should be investigated thoroughly for foci of gonococcal infection. If thorough repeated examinations fail to disclose any evidence of a gonococcal infection, the complement-fixation test should be made several times. If the positive reaction disappears spontaneously, the original positive reaction may be disregarded. If it persists, a further painstaking study of the case for gonococcal activity remains warranted.

Cases with serum reactions of weak intensity. (Two plus—two plus to one plus—one plus, in the first and second tubes respectively)

Serums of forty of the patients studied, or 4 per cent of the series, gave gonococcus complement-fixation reactions of weak intensity. A study of the clinical histories of these patients showed that while the larger percentage had a diagnosis bearing upon the gonorrheal infection (mostly acute urethritis), non-specific reactions began to make their appearance to a noteworthy extent. Thus, six cases were diagnosed as no disease found, though three of these patients admitted having had gonorrheal infections five years prior; three cases were diagnosed as non-syphilitic penile lesions, and were free from clinical evidence of urethral involvement; one non-syphilitic, alcoholic pellagra; two, syphilis, suspected, not proved, no urologic involvement clinically.

Twelve reactions, or 30 per cent of this subgroup, accordingly, must be referred to as non-specific. Upon repeating tests some of these weakly positive reactions might prove to be transient. However, from the view-point of routine study, the impression was gained that complete reliance upon the specificity of the gonococcal complement-fixation reaction must be limited to tests of the strong and moderately strong intensity of fixation.

Cases with doubtful serum reactions. (One plus—trace; one plus—negative; trace—trace; trace—negative; in the first and second tubes respectively)

Serums of fifty of the patients studied yielded gonococcus complement-fixation reactions which are usually designated as doubtful. The diagnoses of these cases are tabulated in table 4.

It is seen from table 4 that thirty-two cases, or 64 per cent of this group, were entirely free from active gonococcal infection. This is more than double the percentage of non-specific reactions found in the group just preceding. The percentage of non-specific reactions of doubtful and weak intensity might be found to be considerably greater among patients of polyclinics. In routine gonococcus complement-fixation, therefore, weakly posi-

TABLE 4

| DIAGNOSIS | NUMBER OF CASES |
|--|-----------------|
| Gonorrheal urethritis, chronic..... | 5 |
| Gonorrheal urethritis, acute..... | 7 |
| Gonorrheal urethritis, posterior | 2 |
| Stricture of the urethra | 2 |
| Prostatitis, chronic | 2 |
| Non-venereal | 32 |

tive reactions should be reported as doubtful, unless the reactions persist, and doubtful results should be reported as negative. The clinician must bear in mind, however, that negative gonococcus complement-fixation in patients suspected of having an active gonococcal focus are of no more diagnostic value than negative routine serologic results are in the cases of suspected syphilitic patients. It is regrettable, yet true, that in both instances the laboratory still fell short of its aim, and the burden of decision fell entirely upon the clinicians.

SUMMARY AND DISCUSSION

In evaluating the study presented in this paper, we are lead to conclude that in routine clinical work gonococcus complement-fixation results are biologically specific only when the intensity of

the reactions is strong or moderately strong. Weak and doubtful reactions are non-specific to a very high degree. Furthermore, in some cases of syphilis of long standing even strongly positive gonococcus complement-fixation reactions appear. This is in disagreement with the opinion of Kolmer³ who believes that in gonococcus complement-fixation "positive reactions including the weakly positive ones are invariably true and specific." Barringer, Strauss and Crowley¹ think that in gonococcus complement-fixation "positive complement-fixation test readings, especially three plus and four plus and over, have definite clinical value which can be relied on." They state further that even a two plus reaction in the later subacute and chronic stages generally means "a full blown, typical gonorrheal invasion which is running its usual course". . . . and "as the clinical picture subsides and the bacteriologic findings become negative, the complement-fixation drops to a one plus reading, then to plus or minus, to minus trace, to minus." Accordingly, the authors evaluate a plus or minus reading as a very weakly positive finding.

Such a method of evaluating the results of gonococcal complement-fixation, when a mixed suspension of the organisms is used, is most probably justified in a follow up study of known cases of gonorrhea that are hospitalized. However, when using the same complement-fixation procedure as a routine on the admission of cases in an outpatient department for the purpose of "picking up" those patients who show no clinical evidence of a gonococcal infection, the evaluation of the laboratory results reported by Barringer and her collaborators will, in our opinion, prove hazardous.

A large percentage of cases with acute gonococcal infection failed to yield positive fixation reactions. A few instances of posterior urethritis likewise gave negative complement-fixation reactions with the unwashed gonococcal antigen. It is evident, therefore, that in the early stages of the gonococcal infection negative complement-fixation results are of no value. This seems to be true equally of cases with the first or repeated infections. On the other hand, persistent strong or moderately strong complement-fixation reactions may be safely regarded as indicative of

residual active infection, or of latent foci of the epididymis, prostate, vesicles, etc. Opposed to this is the wide spread view that about 25 per cent of cases clinically free from any manifestation of the gonococcal disease, regarded as cured, yield positive reactions for varying periods of time and some times indefinitely. The analysis presented in this paper fails to substantiate such an opinion.

Our belief that persistent strongly positive or moderately positive reactions are indicative of residual gonococcal activity or of latent foci is supported by the opinion of Pelouze,⁵ who states that

in repeated infections by the same *strain* there results either an antibody balance or a local toxin desensitization which makes possible for the germ to remain viable for long periods without the other evidence of infection.

Our opinion is further supported by Keyes² who states that gonococcus complement-fixation tests "usually remain positive two to six weeks after all clinical evidence of infection has disappeared." He dismisses the idea of the persistence of any complement-fixation reaction for gonococcal antibody which may be induced by specific vaccine treatment. Keyes states further that a more persistent positive reaction in authentically cured cases is "extremely unusual." So far as the effect of specific vaccine therapy upon the gonococcus fixation is concerned, it need not be considered in our study, since none of the patients whose serums were used received such therapy.

Cases of syphilis previously treated to negative serology show no cross-fixation with the beef heart antigen following the patient's infection with gonococci. Nor is there any evidence appearing in our study indicating extensive cross-fixation of new syphilitic infections with the gonococcal antigen. There is, therefore, truth in the contention of Neuberg⁵ who thinks it is doubtful whether the positive Wassermann reaction has any effect on the outcome of the gonorrhea complement-fixation reaction. Since, however, in 4 to 5 per cent of the total cases studied by us gonococcal complement-fixation occurred with the serums of patients having positive tests for syphilis, but no clinical evidence of the gonococcal activity, the statement of Neuberg must be qualified.

In the light of our experience, the qualifying statement should be as follows: the positive reactions for syphilis appear to have no effect upon the outcome of the gonococcal complement-fixation tests, except in some instances of sero-positive syphilis of long standing.

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A MICRO METHOD FOR THE QUANTITATIVE ESTIMATION OF THE PROTEINS OF BLOOD PLASMA*

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This method for fractionation of proteins in blood plasma was developed for use with small laboratory animals when repeated estimations were desired. Howe's² micro method requires 3 cc. of plasma or serum for complete fractionation. For the refractometric method of Robertson³ 0.5 cc. of serum is needed; this method allows only the determination of albumin and globulin. Wu's⁵ colorimetric method, based on a determination of the tyrosine content of the various proteins, requires 2 cc. and estimates only fibrin, albumin and globulin. Theorell and Widström⁴ have recently published a micro method which reduces the total amount of plasma used to a little over 1 cc., but also is open to the objection that only fibrin, albumin and globulin are determined. The method offered here is based on Berglund's¹ modification of Howe's method and requires 0.7 cc. of plasma for complete fractionation or 0.4 cc. for the determination of fibrinogen, globulin and albumin.

MICRO METHOD

1.5 cc. of blood from the ear of a rabbit is allowed to drip directly into a centrifuge tube containing powdered potassium oxalate, with which it is thoroughly mixed. The tube is centrifuged at 3500 revolutions a minute for four minutes. The plasma is syphoned off through a fine glass syphon into a small straight sided vial containing a 2-holed rubber stopper through one hole of which passes the syphon, and through the other, a short glass tube connected by rubber tubing to a glass mouth piece. By this arrangement the stream of plasma through the syphon can be regulated and the plasma removed from the

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mass of cells to almost the last drop. The plasma is then distributed to previously prepared 10 cc. volumetric flasks by means of a Folin tube for micro determination of blood sugar, accurately graduated in 0.1 and 0.2 cc. graduations.

Preparation of flasks: The following amounts of anhydrous Na_2SO_4 are weighed on an analytical balance into a series of 10 cc. flasks: (1) none (2) 1.07 gm. (3) 1.42 gm. (4) 1.78 gm. (5) 2.204 gm. Into nos. 2, 3, 4 and 5 are added 0.4 cc. of a buffer solution to be described below. To all five is added water at 35° to just below the 10 cc. mark. The flasks are stored in an incubator at 35° in order to maintain the Na_2SO_4 above its eutectic point. When ready for use, 0.1 cc. plasma is measured into each of nos. 1, 2 and 3, and 0.2 cc., into nos. 4 and 5, the volumes made up to 10 cc. with warm water and the flasks returned to the incubator for three hours.

Composition of buffer (Berglund): 29.6 cc. of 0.2 M carbonate free NaOH, 50 cc. 0.2 M of KH_2PO_4 , distilled water (carbonate free) to 100 cc. This should give a solution with pH 7.0.

At the close of the three hour period, the contents of flasks nos. 2, 3, 4 and 5 are filtered. A fine smooth filter paper 6 cm. in diameter is employed. The filtrates are collected in graduated centrifuged tubes; 9 cc. can usually be procured. At the same time 9 cc. of the contents of flask no. 1 are transferred to centrifuge tube no. 1. To each of the five tubes is added 1 cc. of 50 per cent trichloroacetic acid and the contents thoroughly mixed by means of a fine glass rod which is then washed off with 4 per cent trichloroacetic acid. The centrifuge tubes are allowed to stand for ten minutes at room temperature, and are immersed for ten minutes in a water bath at 50°. They are centrifuged at 3500 revolutions per minute for twenty minutes, after which the supernatant liquid is decanted and the precipitate dissolved in one or two drops of 10 per cent NaOH stirring thoroughly with a fine glass rod and spreading the NaOH over the entire inner surface of the centrifuge tube. The contents are then made up accurately to the original volume (9 cc.).

The nitrogen of each of these solutions is determined by Folin's micro kjeldahl method, employing 2 cc. of each. Occasionally 3 cc. may be required for some of the lower fractions of globulin.

$$\text{Computation: } S \times \frac{20}{R} \times \frac{10}{X} \times \frac{100}{Y} = N$$

where S = value of standard, R = reading of colorimeter, X = 0.1 or 0.2 cc., according to the fraction being ashed, Y = no. of cc. ashed, and N = milligrams protein nitrogen in 100 cc. of plasma. To obtain the grams of protein per 100 cc. of plasma, multiply by 6.25 and divide by 1000. Total protein equals protein in tube 1; fibrinogen equals tube 1 minus tube 2; euglobulin equals tube 2 minus tube 3; pseudoglobulin I equals tube 3 minus tube 4; pseudoglobulin II equals tube 4 minus tube 5; albumin equals tube 5. In case values of only total protein, fibrinogen, globulin and albumin are desired tubes 3 and 4 are omitted.

TABLE FOR COMPUTING PROTEIN IN PLASMA

(Grace Medes and Lloyd Medes)

Grams of protein in 100 cc. plasma, computed from formula $0.15 \times \frac{20}{R} \times \frac{10}{0.1} \times \frac{100}{2} = N$ when 0.15 is value of standard in milligrams; R is reading of colorimeter with standard set at 20; when 0.1 cc. of plasma is diluted to 10 cc., and 2 cc. of the filtrate are asked for nesslerization.

| R | N | R | N | R | N | R | N | R | N |
|-------------|---|-------------|---|-------------|---|-------------|---|-------------|---|
| 12.1 = 7.75 | | 15.1 = 6.21 | | 18.1 = 5.18 | | 21.1 = 4.44 | | 24.1 = 3.89 | |
| 12.2 = 7.68 | | 15.2 = 6.17 | | 18.2 = 5.15 | | 21.2 = 4.42 | | 24.2 = 3.87 | |
| 12.3 = 7.62 | | 15.3 = 6.13 | | 18.3 = 5.12 | | 21.3 = 4.40 | | 24.3 = 3.86 | |
| 12.4 = 7.56 | | 15.4 = 6.09 | | 18.4 = 5.10 | | 21.4 = 4.38 | | 24.4 = 3.84 | |
| 12.5 = 7.50 | | 15.5 = 6.05 | | 18.5 = 5.07 | | 21.5 = 4.36 | | 24.5 = 3.83 | |
| 12.6 = 7.44 | | 15.6 = 6.01 | | 18.6 = 5.04 | | 21.6 = 4.34 | | 24.6 = 3.81 | |
| 12.7 = 7.38 | | 15.7 = 5.97 | | 18.7 = 5.01 | | 21.7 = 4.32 | | 24.7 = 3.80 | |
| 12.8 = 7.32 | | 15.8 = 5.93 | | 18.8 = 4.99 | | 21.8 = 4.30 | | 24.8 = 3.78 | |
| 12.9 = 7.27 | | 15.9 = 5.90 | | 18.9 = 4.96 | | 21.9 = 4.28 | | 24.9 = 3.77 | |
| 13.0 = 7.21 | | 16.0 = 5.86 | | 19.0 = 4.93 | | 22.0 = 4.26 | | 25.0 = 3.75 | |
| 13.1 = 7.16 | | 16.1 = 5.82 | | 19.1 = 4.91 | | 22.1 = 4.24 | | 25.1 = 3.74 | |
| 13.2 = 7.10 | | 16.2 = 5.79 | | 19.2 = 4.88 | | 22.2 = 4.22 | | 25.2 = 3.72 | |
| 13.3 = 7.05 | | 16.3 = 5.75 | | 19.3 = 4.86 | | 22.3 = 4.20 | | 25.3 = 3.71 | |
| 13.4 = 7.00 | | 16.4 = 5.72 | | 19.4 = 4.83 | | 22.4 = 4.19 | | 25.4 = 3.69 | |
| 13.5 = 6.94 | | 16.5 = 5.68 | | 19.5 = 4.81 | | 22.5 = 4.17 | | 25.5 = 3.68 | |
| 13.6 = 6.89 | | 16.6 = 5.65 | | 19.6 = 4.78 | | 22.6 = 4.15 | | 25.6 = 3.66 | |
| 13.7 = 6.84 | | 16.7 = 5.61 | | 19.7 = 4.76 | | 22.7 = 4.13 | | 25.7 = 3.65 | |
| 13.8 = 6.79 | | 16.8 = 5.58 | | 19.8 = 4.73 | | 22.8 = 4.11 | | 25.8 = 3.63 | |
| 13.9 = 6.74 | | 16.9 = 5.55 | | 19.9 = 4.71 | | 22.9 = 4.09 | | 25.9 = 3.62 | |
| 14.0 = 6.70 | | 17.0 = 5.51 | | 20.0 = 4.69 | | 23.0 = 4.08 | | 26.0 = 3.61 | |
| 14.1 = 6.65 | | 17.1 = 5.48 | | 20.1 = 4.66 | | 23.1 = 4.06 | | 26.1 = 3.59 | |
| 14.2 = 6.60 | | 17.2 = 5.45 | | 20.2 = 4.64 | | 23.2 = 4.04 | | 26.2 = 3.58 | |
| 14.3 = 6.56 | | 17.3 = 5.42 | | 20.3 = 4.62 | | 23.3 = 4.02 | | 26.3 = 3.56 | |
| 14.4 = 6.51 | | 17.4 = 5.39 | | 20.4 = 4.60 | | 23.4 = 4.01 | | 26.4 = 3.55 | |
| 14.5 = 6.47 | | 17.5 = 5.36 | | 20.5 = 4.57 | | 23.5 = 3.99 | | 26.5 = 3.54 | |
| 14.6 = 6.42 | | 17.6 = 5.33 | | 20.6 = 4.55 | | 23.6 = 3.97 | | 26.6 = 3.52 | |
| 14.7 = 6.38 | | 17.7 = 5.30 | | 20.7 = 4.53 | | 23.7 = 3.96 | | 26.7 = 3.51 | |
| 14.8 = 6.33 | | 17.8 = 5.27 | | 20.8 = 4.51 | | 23.8 = 3.94 | | 26.8 = 3.50 | |
| 14.9 = 6.29 | | 17.9 = 5.24 | | 20.9 = 4.49 | | 23.9 = 3.92 | | 26.9 = 3.49 | |
| 15.0 = 6.25 | | 18.0 = 5.21 | | 21.0 = 4.46 | | 24.0 = 3.91 | | 27.0 = 3.47 | |

This method has been checked against the corresponding macro method in which 1 cc. of plasma is used for each fraction, and found to check within the limits of error of the macro method.

SUMMARY

A micro method for estimation of the protein fractions of plasma is described. For complete fractionation 0.7 cc. of plasma is required. For fractionation into fibrinogen, albumin and globulin, 0.4 cc. of plasma is used.

The method depends upon fractional salting out with anhydrous Na_2SO_4 , precipitating the protein remaining in the various filtrates with trichloroacetic acid, dissolving the precipitates in NaOH and determining the nitrogen in an aliquot part.

The error of the determination is not greater than that of the corresponding macro method.

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GELATINOUS CARCINOMA OF THE BREAST

SECOND REPORT

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In a previous communication¹ on this subject it was regreted that the case reported at that time did not permit a description of the histology of the metastases. For that reason the following case report is considered important.

CASE REPORT

A radical amputation of the left breast was performed on a woman, aged 59, January 11, 1933. The clinical history revealed that three months prior to operation the patient was struck over the left breast; the blow was not severe. Several weeks later she noticed that the nipple was firm and appeared to be retracted. Subsequent to this she noted a small growth in the left breast which she treated with poultices. This growth progressively enlarged and soon was the seat of severe throbbing pain. Shooting pains into the left axilla were noticed upon movement of the left arm. There has not been any discharge from the nipple. There was no loss of weight. Past history, previous illnesses, family history were of no consequence.

Physical examination revealed that the right breast was free of palpable tumor masses, but that a hard, irregular, tender mass was present in the outer, lower quadrant of the left breast close to the nipple. It was not freely moveable. The nipple was retracted, not discharging. The skin over the tumor was somewhat reddened and nodular. There was a large, tender gland in the left axilla about the size of a walnut. The pre-operative diagnosis was: carcinoma of the left breast with extension to the axilla.

Pathological Report: "The specimen consists of the left breast, pectoral muscles and axillary fat tissue. The nipple is depressed, slightly bluish in color. There is a crescentic elevation around the margin of the nipple, so that the normal groove between the nipple and skin is effaced. Section through the longitudinal axis in the nipple line reveals a non-encapsulated, distinctly colloid or gelatinous tumor about 2.5 cm. in diameter. It is located in breast tissue and extends upward into the nipple zone. This upper portion is more distinctly

of a tumor character, homogeneous, gray tissue, quite lacking in colloid material. The colloid structure of this tumor is so striking as to warrant the descriptive term "honey-comb." In the axillary fat a large nodule of similar tissue is found, cross section of which reveals a distinctly neoplastic, gelatinous-filled structure. The microscopic examination reveals a cellular, rather anaplastic, papillary growth, in which there are numerous mitotic figures and irregular invasion of the stroma (Figure 1). In the colloid areas the cells are disrupted and atrophic. One may trace the origin of the colloid material in both the axillary mass and the

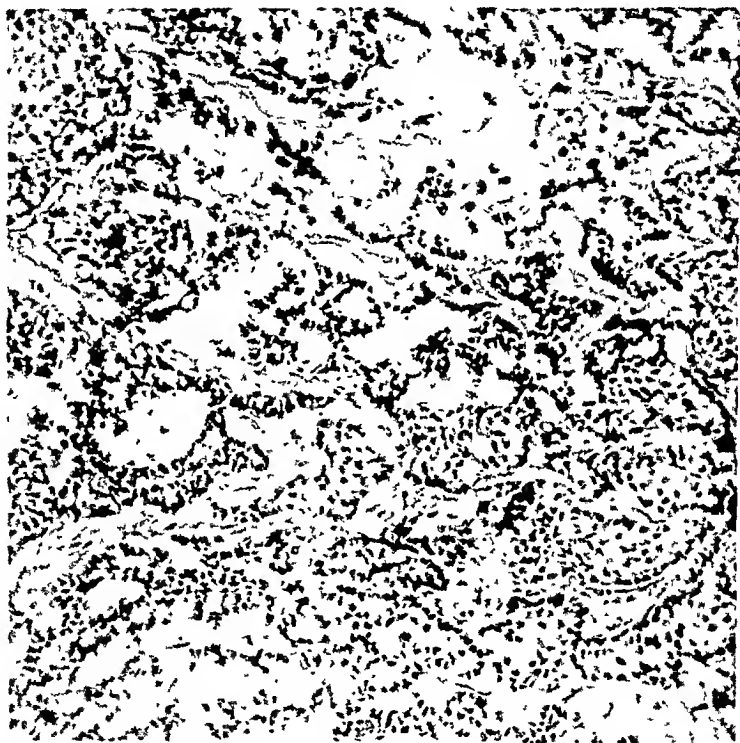


FIG. 1. METASTATIC AXILLARY LYMPH NODE

primary tumor to the cells themselves. The cytoplasm seems to have filled up with this material to the point of cell rupture, whereupon the stroma was filled with this material and the cells either destroyed or so compressed as to be hardly distinguishable."

This case illustrates the mechanism of colloid change in carcinoma of the breast and offers striking proof that the process is epithelial in origin. The nature of this process would seem to be

one of intracellular metabolism. Gelatinous carcinoma of the breast should be considered as possessing peculiar and destructive qualities which are carried with the cells and reproduced in the metastases.

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A SIMPLE METHOD WITH A NEW APPARATUS FOR RAPID DIALYSIS*

PRELIMINARY REPORT

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P. LeComte du Noüy^c reached an interesting conclusion from his experiments on the surface tension of proteins which he states as follows:

If we attempt to calculate the size of the vessels which would require pure serum to develop such a monolayer . . . it is found that they would have to be of the order of magnitude of the blood capillaries, filled with blood cells. Thus one of the factors governing the concentration of colloids in the serum is perhaps found."

This conclusion led one of us (I. A. N.) to speculate on the possibility of studying fresh uncoagulated blood in vessels approaching the capillary size, or the whole blood with its proteins approaching the monolayer state. No way to make capillary tubes seemed practical so the possibility of spreading the blood very thin was tried. No monolayer studies are reported at this time but experiments indicate that this apparatus might be of practical use in routine procedures in medical, industrial, and research laboratories. The data included in this report are merely for the purpose of illustrating the rapidity and reliability of the apparatus.**

It was found that by putting 0.2 cc. of blood on the convex surface of a large watch glass and spreading it out by means of a

* Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

** At the author's suggestion, the Central Scientific Company is considering the development of a suitable commercial model.

piece of wet cellophane stretched tight on an embroidery hoop 17.8 cm. (7 inches) in diameter, a spread of 15.3 cm. could be obtained. Calculations showed that the thickness of the blood layer was about 6 microns. It is evident that the total surface achieved (area of spread plus the area of all the erythrocyte surfaces) enables one to approach a monolayer state of the proteins much closer than with a collodion sac or similar dialyzer.

Tests for blood sugar at this time showed (by Folin's ferricyanide method) that in two minutes dialysis and equilibrium were complete when using 0.2 cc. of blood, 9.8 cc. of water and a dry cellophane.

The tests were repeated with larger frames and larger amounts of water and varying amounts of blood. When 4 cc. of serum with a spread of about 20 cm. and 46 cc. of distilled water were used, equilibrium was almost complete in twenty minutes. These tests showed roughly the relationship of surface, blood thickness, amount of diluent, and time in attaining an equilibrium with this apparatus. They also showed that by choosing proper curvatures a relatively large amount of blood could be held in contact with the dialyzer, even though the blood was not in a retaining vessel.

Up to this time rubber bands had been used to hold cellophane on the frame. Sometimes the rubber bands held so firmly that the cellophane cracked in drying. Also, on rewetting, the cellophane tended to sag and the slack had to be taken up by gently pulling on the edges. It became apparent that some way would have to be devised whereby cellophane could be held firmly stretched when wet and yet not crack when dried on the frame.

The following prerequisites were found necessary:

(1) The wetted cellophane must be fastened with enough sag to allow for a difference of from 2.5 cm. to 4.0 cm. for each 30 cm. in expansion and contraction when in the wet and dry states.

(2) The cellophane should not dry in contact with rubber or any other surface except at the circumference because it tends to adhere and when wet again, to crack. Even if no cracking occurs it tends to peel off a scum from the rubber making the dialysate solution turbid.

(3) Visibility of the colloid must be maintained to insure uniform spreading during manipulation of the apparatus.

Various forms of apparatuses were devised which would overcome these difficulties. In addition such accessories as vacuum and pressure control, temperature regulation, continuous operation, and electrodialysis were devised. But finally special efforts were made to construct a simple model which any technician could assemble and use with a minimum of expense and mechanical ingenuity. Since it is not necessary to have a dried cellophane for all routine work, two models are suggested.

DESCRIPTION OF APPARATUS FOR DRIED CELLOPHANE

A model for the use of cleaned and dried cellophane requires and is made as follows:

(1) A grooved rim on which to fasten a continuous rubber tube and the cellophane. A metal wheel about 24 cm. in diameter and 0.6 to 1.3 cm. wide (such as come on baby carriages) is satisfactory.

(2) A continuous rubber tube with an air vent is formed over the above rim. (The Miller Rubber Products Company made some rubber bladders about 21 cm. wide and 25 cm. long, with a vent on the 21 cm. side—such as they make on rubber toys.) The 25 cm. sides are open or should be trimmed so one has in effect not a bladder but a large open rubber tube. Stretch one open end evenly over the above rim and tie on with a string. Now overlap the other end of the tube over the first end so that the rim is entirely within a continuous tube with the vent 2.5 cm. from the edge. When the rubber is so adjusted that it is even, with a central hole about 18 cm. in diameter, tie firmly. A little rubber glue may be smeared between the two layers of rubber by reflecting the overlap so a space over the rim and into the groove may be reached. The finished frame now looks like a flattened rubber doughnut which can be inflated. The continuous rubber tube enables one to allow a sufficient expanse of wet cellophane so it will not crack when dry and provides a mechanism for holding it stretched when wet again by simply inflating.

(3) A base is used on which to place the colloid solution. Any object with a convex surface may be used. We have used large watch glasses and lids from kitchen utensils. These may be paraffined. However, much more control may be obtained if the convexity can be adjusted. We have found that a rubber surface does not interfere with the blood tests so far done except possibly in the case of calcium. We are using a 25 cm. cake pan with a sheet of rubber stretched over the top and tied over the edge. An air vent is soldered into the side of the pan.

(4) A cover is convenient to keep out dirt and lessen evaporation if one is interrupted and has to let the set up stand for some time. A large watch glass, large funnel, or inverted vessel may be used.

To fasten the cellophane on the frame the tube is inflated to about 5 cm., the cellophane thoroughly wetted and carefully smoothed over the groove of the apparatus. If a small rubber band is slipped over the cellophane and into the groove, the cellophane may be easily smoothed of any sharp wrinkles as it seems to be somewhat plastic when wet. The cellophane may be fastened on firmly by using a sufficient number of rubber bands, or tied on. A rubber band as wide as the rim may be used and the string tied over this and thus remain dry. All excess cellophane should be trimmed close to the tie as cellophane tends to act as a wick, introducing possible contamination from the outside or loss of some dialysate solution.

To dry the cellophane the rubber tube is deflated completely. This draws the rubber away from the plane of the cellophane thus allowing it to be kept dry for any length of time without sticking to the rubber. While drying, the dialyzer may be suspended by clips fastened to the excess overlap.

METHOD OF USING THE DIALYZER

(1) Place the dialyzer on the base for convenience in handling and put the dialyzing fluid in the dialyzer. Distribute it so that all the cellophane becomes thoroughly wet in a short time with less evaporation.

(2) Now inflate the rubber tube until the cellophane is stretched.

(3) Inflate the rubber drum of the base and place the colloidal solution to be analyzed on the dome. It will not run off.

(4) Gently lower the dialyzer on the colloidal solution. By raising and lowering, or rotating the dialyzer, the colloidal solution may be teased to wet the cellophane in wider circles in a few seconds. The slower the blood is spread, the thinner will be the layer, just as in making blood smears for differential counts. The drum may now be deflated until plane and thus get an even distribution of the dialyzing solution in the dialyzer. The dialysis seems to reach rapid equilibrium without further manipulation.

If a dialysis is desired and the dialyzer is not dry, it may be washed and used in a manner to be described under the wet method.

DESCRIPTION OF A METHOD FOR USING WET CELLOPHANE

All that is needed is a bell jar of the Graham type or a suitable frame on which to tie the cellophane. Thoroughly wet a sheet of cellophane and stretch it smoothly over the frame, tying with string or using several rubber bands. Wash and drain off the excess water. A 25 cm. dialyzer takes up roughly about 3 cc. of fluid, so if a 1 in 20 dilution is wanted, add 16 cc. of fluid when 1 cc. of colloidal solution is used. Dialyze as with the dry method, manipulating the coaptation of the cellophane to the surface of the base with the colloidal solution between them. After equilibrium is established, remove a measured quantity of the dialysate for the determinations and add a measured quantity of a colored dialyzable solution such as alkalinized phenolphthalein from a stock on

hand. After equilibrium is reached with this (ten minutes seems sufficient), remove some of the now colored dialysate in the dialyzer and compare in the colorimeter against the stock colored solution. Calculate the water originally present in the cellophane. After using this dialyzer, it may be washed and set in some appropriate space in the laboratory, with water in the dialyzer to avoid drying and breaking of the cellophane. Recent preliminary tests indicate that a few drops of glycerol spread out on the wet cellophane keep it moist for weeks without cracking and inhibit the growth of fungi.

REPORT ON SOME EXPERIMENTS

A patient was admitted to the Hospital with very high non-protein nitrogen blood constituents. We had found it more satisfactory and accurate to use from 19 cc. to 49 cc. of water in a 25 cm. dialyzer to 1 cc. of blood on the base. These ratios

TABLE 1
COMPARISON OF VALUES
(Mgms. per 100 cc).

| METHOD | NON- PROTEIN NITROGEN | UREA NITROGEN | URIC ACID | CREAT- ININE |
|----------------|-----------------------------|------------------|--------------|-----------------|
| Dialysate..... | 190.0 | 141.5 | 8.1 | 13.5 |
| Filtrate..... | 193.0 | 141.5 | 7.9 | 13.5 |

permitted us to use our routine reagents for the high values for non-protein nitrogen substances in this patient's blood. For the regular determinations the filtrates were diluted accordingly. The precipitations were started at the same time as the dialyses. Ten minutes were allowed for the dialyses to reach an equilibrium. The same standards were used for the dialysates and the filtrates (see table 1).

These and other tests have convinced us that this dialysate method is at least as fast and as accurate as precipitation and filtration for the separation of free crystalloids from the colloids of the blood.

GENERAL REMARKS

The apparatus is readily washed. Soak the cellophane and base with a weak sodium bicarbonate solution, rinse and dry.

Soap may be used. Gortner² quoted Brinkman and Szent-Georgyi to the effect that sodium oleate will make a celloidion sac permeable to hemoglobin at three atmospheres pressure. We have found no significant alteration in permeability in ten minutes with cellophane that had been washed with ivory soap and that had been rinsed thoroughly.

We have used Congo red to test for leaks. Michaelis⁴ reported that protein may take Congo red through an ultrafilter which (due to surface charge) would not permit Congo red alone to pass through. We have left Congo red mixed with serum on the dialyzer for hours and could not detect any Congo red color in the dialysate from cellophane #300, even after concentrating a quantity of dialysate. Starch has been used by Aitken¹ to test the permeability of celloidion sacs. We have left starch on the dialyzer for hours and could not detect any color in the dialysate with the iodine test.

Cellophane #300 has been used because it has been tested by various workers and found to be impermeable to proteins. Nicholas⁵ used 200 lbs. pressure for one and three-fourth hours and found no proteins in the ultrafiltrate. Cellophane is readily obtained, is uniform, is cheap, and we have found that by the method presented in this paper it may be used repeatedly. However, the apparatus and methods are adaptable to other membranes which might be used or developed with a consideration for physical or chemical factors.

Freshly drawn whole blood has been used without anticoagulants, in some tests. It appears that the free calcium is lost so rapidly that together with a certain amount of defibrination which seems to occur, no coagulation takes place.

Most of the laboratory manuals on colloid chemistry describe various types of dialyzers. Some of these exhibit a large amount of surface with a small amount of liquid to be dialyzed—such as Pauli's folded parchment paper.³ There have also been a number of methods reported in recent years using celloidion sacs for rapid dialyses. So far we have not found any apparatus using cellophane or other membrane repeatedly in an apparatus or method such as I have presented.

SUMMARY

Two models of an apparatus using cellophane #300 and a method of obtaining certain blood dialysates at least as rapidly and accurately as blood filtrates, have been described.

It is possible to spread blood with its erythrocytes in such a thin layer with this apparatus that the proteins probably approach a monolayer state.

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REMOVAL OF THE SPINAL CORD BY THE ANTERIOR ROUTE: A NEW POSTMORTEM METHOD

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Removal of the spinal cord and its membranes by reflecting the skin and muscles of the back, and removing the laminae and spinous processes of the vertebrae has been a laborious task, and has retarded satisfactory study of this structure and its appendages. The modification of this technic which is the subject of this note has been used in the laboratories of Pathologic Anatomy of The Mayo Clinic for eight years, and has been so satisfactory and comparatively simple that it seemed to me to be worth recording.

No elaborate equipment or instruments are necessary, and no new ones are required. A saw with a rounded end, a wooden hammer, and an all steel chisel are used. Part of the standard equipment is a large pair of Councilman's bone cutting forceps* used to remove the inner and middle ears, and formerly used extensively by orthopedists.

After the organs have been removed from the thorax and abdomen, the bodies of the vertebrae are cleaned, and then cut with the saw in the direction indicated in figure 1. The cutting should be commenced at the promontory of the sacrum and then continued upward to the fourth thoracic vertebra or even higher. The pedicles on the right are cut, either at the level of the intervertebral foramina with the chisel, or with Councilman's forceps, and leverage is obtained to remove the segment of vertebra with the large forceps. If assistance is available, a chisel inserted into the cut also pries the segment of vertebra laterally, and assists in its removal. The half body of the vertebra can be re-

* Councilman's Bone cutting Forceps No. A/-300, obtained from The KNY Schierer Company, New York.

moved individually by cutting the intervertebral disks with a knife; this is a simple procedure, and usually advisable; it permits also examination of the cartilage of the intervertebral disks.

When the segments of vertebrae are removed, the anterior surface of the dura mater and the nerve roots (fig. 2), as well as the dorsal root ganglion and nerve trunks, are exposed. The dura mater with the spinal cord within, the nerve roots, and dorsal root ganglia can be removed intact by cutting the nerve roots of the left side, or if desired, the dorsal root ganglia on this side can be easily obtained for examination. The only objection to this method is the possibility of injuring the spinal cord with the saw, but after a little experience, this danger disappears. Another

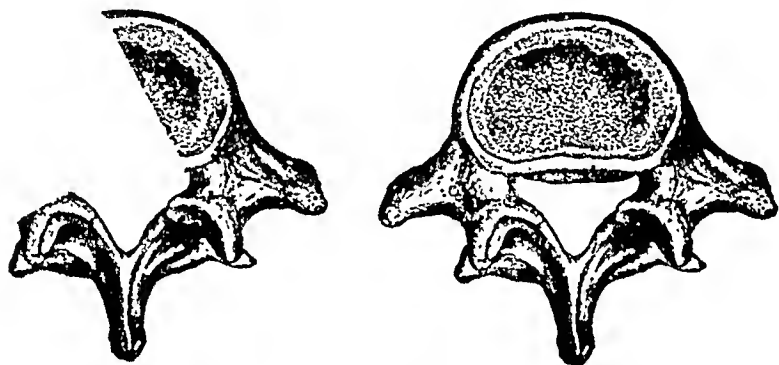


FIG. 1. AMOUNT OF EXPOSURE WHICH CAN BE OBTAINED

possible objection is that the cervical segments of the spinal cord are not exposed or readily obtained, but if the cervical cord is desired a thin knife can be inserted between the occipital bone and the atlas and the medulla cut across. The dura mater at its upper level of exposure is cut completely around, and with gentle traction toward the feet, the entire cord, without the dura mater and dorsal root ganglia can be obtained. The spinal cord secured in this manner is intact and satisfactory for histologic or chemical studies.

The advantages of this method compensate for any possible objections which might be made to it. Special permission to examine the spinal cord is not necessary, special equipment is not

necessary, the body does not have to be turned, and there is less work for the undertakers. Sufficient strength of the vertebral column remains, but if desirable, the defect in the bones can be

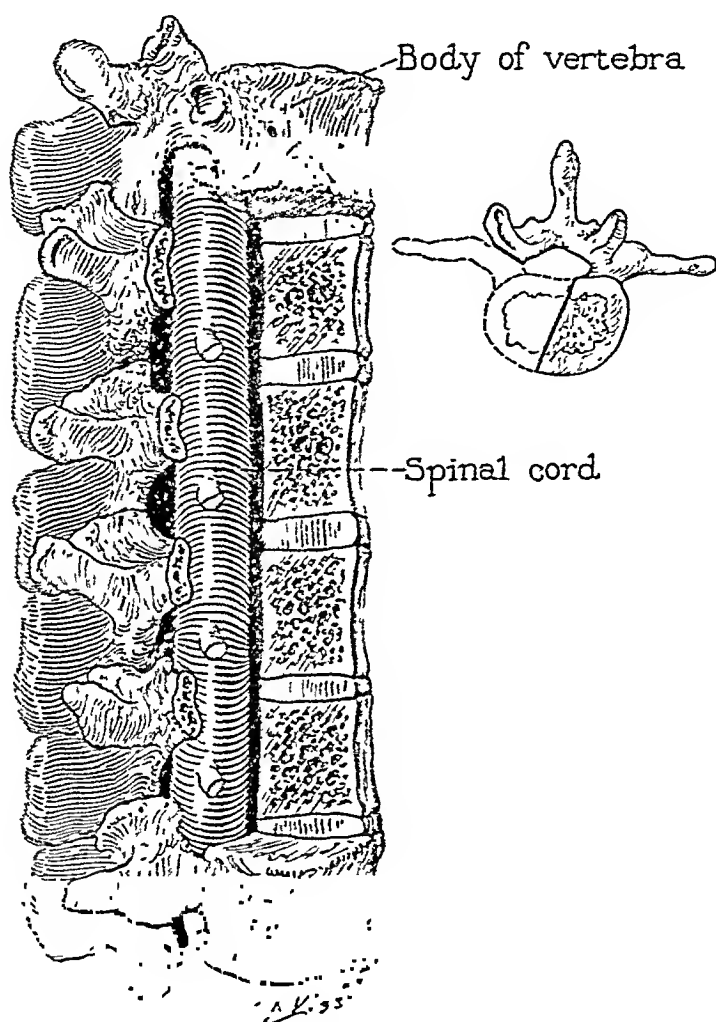


FIG. 2. EXPOSURE OF THE SPINAL CORD SURROUNDED BY THE DURA MATER AS WELL AS THE NERVE ROOTS

The dorsal root ganglia and the nerve trunks can also be removed completely. This exposure can be obtained for the entire spinal cord up to the third thoracic vertebra or even higher.

repaired with plaster of paris. By this method the work can be done more easily and more quickly than by that ordinarily used. In this manner I have obtained for examination several hundred

spinal cords, and Harmeier has used the method exclusively when removing the filum terminale for his study of the normal structure of this tissue. The method is particularly advantageous in obtaining the lumbar portion of the spinal cord, as well as the cauda equina. At this level, it is difficult to approach the spinal cord from the back because of the heavy muscles, the sacro-iliac joint, and the depth of the cauda equina from the surface.

TORULA MENINGITIS*

REPORT OF CASE AND SUMMARY OF LITERATURE

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Medical knowledge of diseases due to parasitic plants is astonishingly recent in origin, the discovery of such minute objects necessarily awaiting the advent of optical magnification. Fungi, being larger than bacteria, were naturally discovered first. Dr. Robert Hooke, of England, using his bubble of glass filled with water as a microscope, in 1656 described a fungus disease of rose leaves, but not until the advent of the compound microscope of two hundred years ago was it possible for Langenbeck to describe the yeast-like cells found covering the oral and pharyngeal mucosa of a cadaver at autopsy, and for Charles Robin to accurately describe "thrush" as a fungus disease in 1843.

Fungi approach and often exceed the size of the average body cell. They are not able to grow and reproduce within the animal body to the unlimited extent that bacteria can. They produce a relatively small amount of exotoxins. Their disease processes are more probably due to mechanical displacement of cells thereby altering normal physiologic processes. For these reasons fungus diseases are usually characterized by great chronicity and very slight infectivity. Probably all of the systemic fungus diseases represent very accidental implantations of microscopic plants which are barely able to survive and reproduce slowly within living animal tissues.

Out of a total of something over 5000 genera of fungi recently listed by Clements and Shear¹ it is significant that there are only twenty-six well recognized groups that can and do produce definite

* Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

disease processes. It is highly improbable that there will be many additions to this list. With the exception of members of the order of Trichomycetes, or "higher bacteria," as many medical writers prefer to call them, medical knowledge is fairly complete in its conception of etiology and pathology. Nomenclature, or rather a superabundance of it, has served to produce a state of utter lack of specific information about these few diseases in the minds of not only medical practitioners but the authors of the majority of our medical laboratory text-books as well. For this reason alone the recognition of systemic fungus infections by otherwise competent physicians and clinical pathologists has just about reverted to the par value of one case per lifetime which was established by Charles Robin some two hundred years ago.

CASE REPORT

Mr. D. L., white male, aged forty-eight years, book-keeper, consulted Drs. Y. Ardoin and C. L. Attaway, of Ville Platte, Louisiana, on Feb. 2, 1932, for relief from a persistent headache. The patient had had recurrent attacks of migraine for a long period and insisted that this particular headache was different, describing it as a "bursting headache," not affected in the slightest by any of his usual remedies. The usual regimen with catharsis and diet gave no relief.

Within two weeks drowsiness, blurred vision, loss of weight and strength were definitely present. He continued work for the next three weeks, finally entering the hospital on March 17.

Complete examination upon his entry into the hospital showed an emaciated patient with slow and interrupted speech. The general physical findings were as follows: a slight but definite cervical rigidity; positive Kernig; tendon reflexes increased generally; negative findings in chest and abdomen; a small granuloma with crusted surface in the right submaxillary region, which was said to have developed from a razor cut; temperature, 99°F., pulse 60, respiration 18; roentgen ray examination of skull, stomach and chest were negative.

The patient alternated between periods of extreme restlessness and prolonged stupor, with eyes closed practically all of the time. When aroused he would only ask for something to relieve the headache, which was only accomplished by the use of morphine.

On March 25 the spinal fluid examination revealed a meningitis caused by a fungus, which we will describe more in detail later. Scrapings from the lesion on the jaw revealed the same type of budding yeast-like fungus found in the spinal fluid. The spinal fluid findings were re-checked on subsequent lumbar puncture for therapeutic relief of pressure.

The patient gradually lost ground, his pulse remained slow and his tem-

perature was usually subnormal. He died on April 10, 1932, following an illness of seventy-three days.

An autopsy was denied. The lesions illustrating the following discussion were produced in rabbits by cisternal injection of the pure cultures obtained from the spinal fluid.

DISCUSSION

Systemic infections by a pigment producing yeast that reproduced by budding only, on living or dead media was described as a clinical entity by Stoddard and Cutler,⁴ of Boston, in 1916. The fungus was determined as a member of the *Torula* group by these workers and named *Torula histolytica* from the characteristic appearance of disintegrated brain tissue surrounding colonies of the fungi. In addition to their two cases and the four cases that they were able to identify in previous literature, we have attempted to obtain a complete list of systemic torular infections by reviewing all apparently pertinent titles appearing in the Cumulative Index of Medicine. Including this case the total number of authentic systemic infections due to torula has reached a total of forty-six. Of this number thirty-one have occurred in the United States and fourteen have been recorded from England, Germany, Australia, Japan, France, Dutch East Indies and Italy. The organism thus apparently lives independently of man, and is world-wide in its distribution.

Clinically, human infections with yeasts of the genus *Torula* usually involve the central nervous system and by preference the meninges (pia and arachnoid). A few instances are on record of small abscess-like areas within the gray matter, or involving only the lungs. The organisms gain entrance into the body chiefly by way of the respiratory tract, although the portal of entry is often not ascertainable. In one instance trauma over the scapula was followed by a deep mycotic infection that had healed many months before the onset of the typical meningitis. This is in direct contrast to systemic infections with *Cryptococcus gilchristi* (blastomycosis) or *Coccidioides immitis*, where the primary lesion almost invariably occurs in the skin. In our present case the initial lesion was proved to be a small superficial granuloma that developed from a razor cut.

The lesions of torulosis will not be found described in any of the texts commonly employed in pathology. Stoddard and Cutler's original description of the two types of central nervous involvement (meningeal or focal within the gray matter of either the cerebrum or the cerebellum) will be found most complete. Freeman² has more recently reviewed this pathology in a survey of all of the various fungous lesions involving the brain or its coverings. His description is complete and well illustrated.

Essentially the torular lesions are chronic inflammatory in type, the organisms consisting of colonies of yeast-like budding fungi lying in areas of liquid or necrotic material. Such a characteristic lesion was shown in rabbits inoculated with cultures from our case which was of the meningeal type (see fig. 4). The organisms present a characteristic zone of retraction (or are surrounded by a clear transparent secretion, as this is occasionally described). The smaller fungi stain rather darkly with Gram's stain, and the larger ones present a definitely stained, thick cell wall with a dark central mass situated rather eccentrically. The appearance of torula is quite characteristically different from the *Cryptococcus gilchristi* (see fig. 6), which has a thick non-stained cell wall, with several dark-staining masses of nuclear material within the body of the fungus, and neither of these resembles the endosporulating type of *Coccidioides immitis* (figs. 7 and 8). All of these illustrations were photographed with the same optical system.

FIGS. 1 TO 8

FIG. 1. Spinal fluid showing type of cells (mononuclears) and fungi (*Torula histolytica*) present.

FIG. 2. Budding forms of fungus found in fresh fluid.

FIG. 3. Multiple budding shown in microcultures.

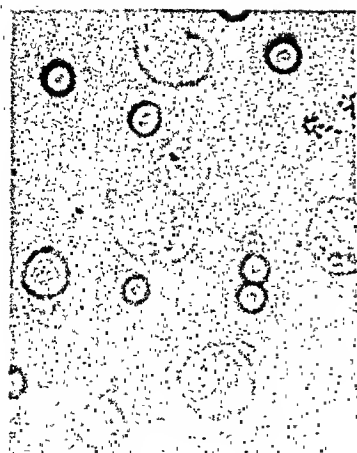
FIG. 4. Chronic inflammatory reaction with edema in the meninges. Dark area is a collection or colony formation in necrotic material shown in Fig. 5.

FIG. 5. *Torula histolytica* present in necrotic material in meningeal lesion (rabbit).

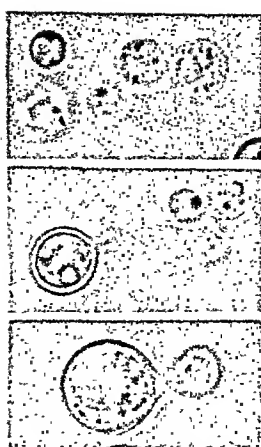
FIG. 6. *Cryptococcus gilchristi* in lesion of lung.

FIG. 7. *Coccidioides immitis* in lesion of lung.

FIG. 8. Endosporulating form of *coccidioides* in lung.



1



2



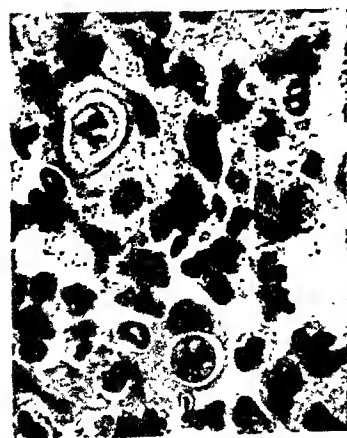
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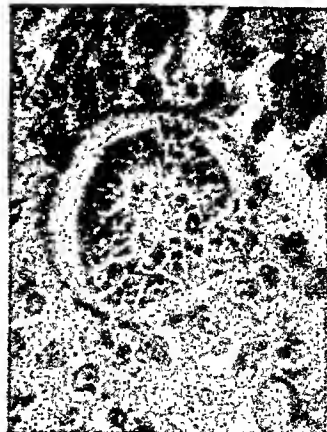
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7



8

FIGS. 1 TO 8

While the great majority of torular lesions have been frankly meningitic in type, only a very few cases have been diagnosed before death by means of cerebrospinal fluid examination. There is a still shorter list of cultural studies of the organism. The spinal fluid of our case was faintly hazy by indirect light, with 650 mononuclear cells and a slightly larger number of torula cells (see fig. 1). The common practice of using the 16 mm. objective for cell counting has undoubtedly contributed largely to the lack of recognition of the infection. Under higher magnification (1/12 oil immersion objective, No. 4 Leitz ocular and a camera bellows of 18 inches, which was also used in photographing figs. 5, 6, 7 and 8) the thick walled budding fungus may be recognized with certainty. The thick, highly refractile cell wall of the larger forms resembles cryptococci (blastomycetes). Microcultures in glucose veal infusion agar in our case soon revealed characteristic multiple budding forms without mycelia. Yellowish pigment production was noticeable on surface agar colonies within a week, during which time none of the common sugar mediums was appreciably fermented. Scrapings from the lesion on the jaw yielded a morphologically identical budding fungus.

Several reported cases have been undoubtedly produced by a non-pigment-forming type of torula. This calls for some further classification of species. Applying Harrison's³ classification of the torulae to the most correctly employed botanical nomenclature of Clements and Shear⁴, we may classify these fungi as follows:

Phylum, Thallophyta; Sub-division, Eumycetes;

Group, Deuteromycetes (Hyphomycetes or Fungi Imperfecti)

Order, Moniliales; Family, Pseudosaccharomyces;

Genera, *Rhodotorula*, producing red pigment.

Chromotorula, producing other pigment.

Torula, producing no pigment.

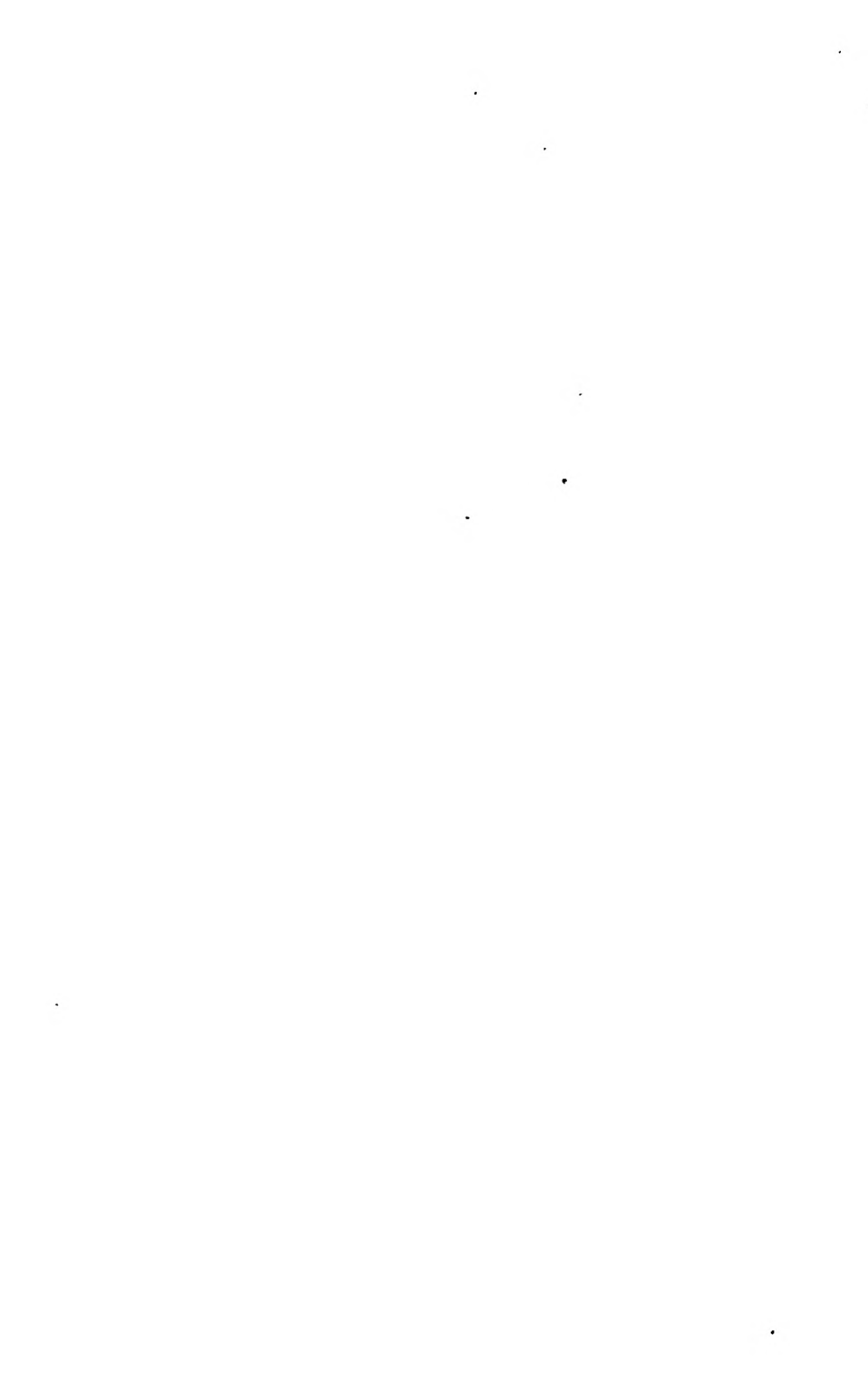
The common name for the fungus discussed in this paper as now used throughout the medical literature is *Torula histolytica*, Stoddard and Cutler, 1916. Unfortunately the designation of *Torula Histolytica* was given by Harrison to one of the very few non-chromogenic cultures of human origin. Much valuable

literature would be lost to students of medicine if teachers and pathologists discard this name.

We should prefer to see some large group of clinical pathologists correlate the commonly known names of the pathogenic fungi into an orderly medical classification and describe accurately and illustrate their undoubted characteristics in tissue or standard cultures. Then by means of a relatively small list of synonyms the medical worker could transfer his findings to some such botanical classification as is attempted by the Saccardo system used extensively abroad, or its interpretation by our own American botanists, Clements and Shear. Until such time as will witness the ability of the average clinical pathologist to determine with a fair degree of certainty such fungi as are commonly found in various human exudates, the number of such cases that escape proper recognition can only be conjectured. It is certain, however, that only a fraction of the actual incidence of such infections is represented by the small number of such cases that appear in the records.

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EDITORIAL

VIRUSES—A NEW ERA IN MEDICINE

The history of modern medicine may be divided into three periods each of which is characterized by the dominant part played by a particular laboratory group. First the morphologists, to whom we are indebted for rescuing medicine from what Garrison calls "fanciful speculation," held the center of the stage until the creation of the wholly new science of bacteriology. Workers in this new field of research contributed so much to the knowledge of the cause of the infectious diseases and to the prevention and cure of some that they compelled the attention of the medical world for many years but were in turn superseded by the bio-chemists who had long been busy laying the foundations for the wonderful discoveries now familiar to all.

There are indications that we have entered a fourth period which is to be dominated by still another laboratory group namely, the investigators of the filterable viruses. These men have shown such imagination, energy, and ingenuity that one does not hesitate to predict that their investigations will result in discoveries comparable to those of the bacteriologists and bio-chemists. Space limitations permit the mention of only a few examples of their work.

Li and Rivers⁸ have demonstrated that some of the viruses can be grown in large quantities in a medium of minced chick embryo and Tyrode Solution whereas until recently the only method available was tissue culture. Goodpasture and his associates⁵ at Vanderbilt University have contributed a brilliant and prize-deserving method in which the chorio-allantoic membrane of chick embryos is used as a medium. They have not only grown the viruses of vaccinia, fowl-pox, and herpes simplex but have studied the reaction of the three types of embryonic cells to them. Stevenson and Butler¹² following Goodpasture's sug-

gestion and using his method have succeeded in growing vaccine virus in large quantities and free from contaminating organisms and claim superiority of the method over that customarily used. Burnet² has employed the same method in the study of canary-pox. The Vanderbilt group¹³ has proved that the virus of fowl-pox is in the cell inclusions associated with the lesions and have gone even further in that they have produced the disease by injecting some of the minute elementary bodies which are found within the inclusions.¹⁴ Amies¹ and Ledingham⁷ have shown agglutination of the elementary bodies of vaccinia and varicella by the serum of patients with these diseases and Parker and Muckenfuss⁹ have performed complement fixation reactions in vaccinia and variola using as antigen the contents of pustules which are known to contain the elementary bodies in large numbers. So much evidence has been brought forward to prove that these bodies cause disease that Goodpasture⁴ has proposed the name *Borreliota* as a generic title and a variety name for each strain thus not only giving a name to these interesting structures but also doing honor to Borrell their discoverer.

While *Borreliota* have been found in many of the virus diseases there are many others in which no particulate matter has been demonstrated, nevertheless much interesting work is being done with these too. Shope¹⁰ has obtained a filterable virus from swine suffering from swine influenza which, in combination with a hemophilus bacillus, produces the disease when applied to the nasal mucosa of healthy animals. Using the virus in the same way but without the bacillus or by injecting it intramuscularly the disease is not produced but a solid immunity is conferred. Smith, Andrewes, and Laidlaw¹¹ have recovered a virus from patients with human influenza which shows a close antigenic relationship to the swine virus. Ferrets are equally susceptible to the two viruses and the serum of an animal recovered from the inoculation of one will protect against the other.

Still another group of diseases is being investigated by the virus workers. Furth³ has produced leukomatosis, myelomatosis, a variety of endothelioma, and at times leukemia, in chickens by means of a filter passing virus and Gordon⁶ has performed experi-

ments which suggest that Hodgkin's disease may belong to the virus group. He finds that rabbits injected intra-cerebrally with emulsions of lymph glands from Hodgkin's disease patients develop a symptom complex not seen when similar emulsions prepared from lymph glands with other lesions are injected, moreover he found small bodies in lymph nodes of patients with Hodgkin's disease and in the brains of the inoculated rabbits, indistinguishable from the elementary bodies found in vaccinia and fowl-pox. Although he has not found the agent to be filterable it resembles the viruses in its behavior to heat and the antiseptics.

This by no means exhausts the subject but enough has been indicated, it is hoped, to justify the thesis that a new era in medicine has come.

W. S. THOMAS.

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